

# Target Validation in Drug Discovery

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# Alzheimer's disease (AD)

Alois Alzheimer (1864-1915), a German psychiatrist and neuropathologist, described the pathology and the clinical symptoms of presenile dementia in 1906

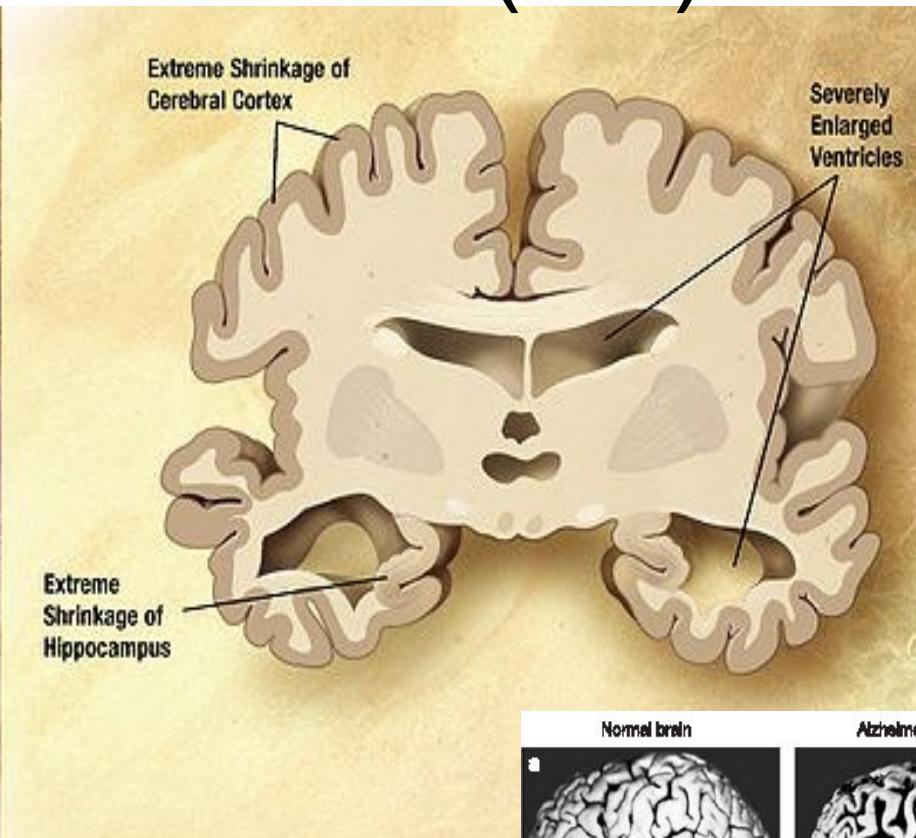
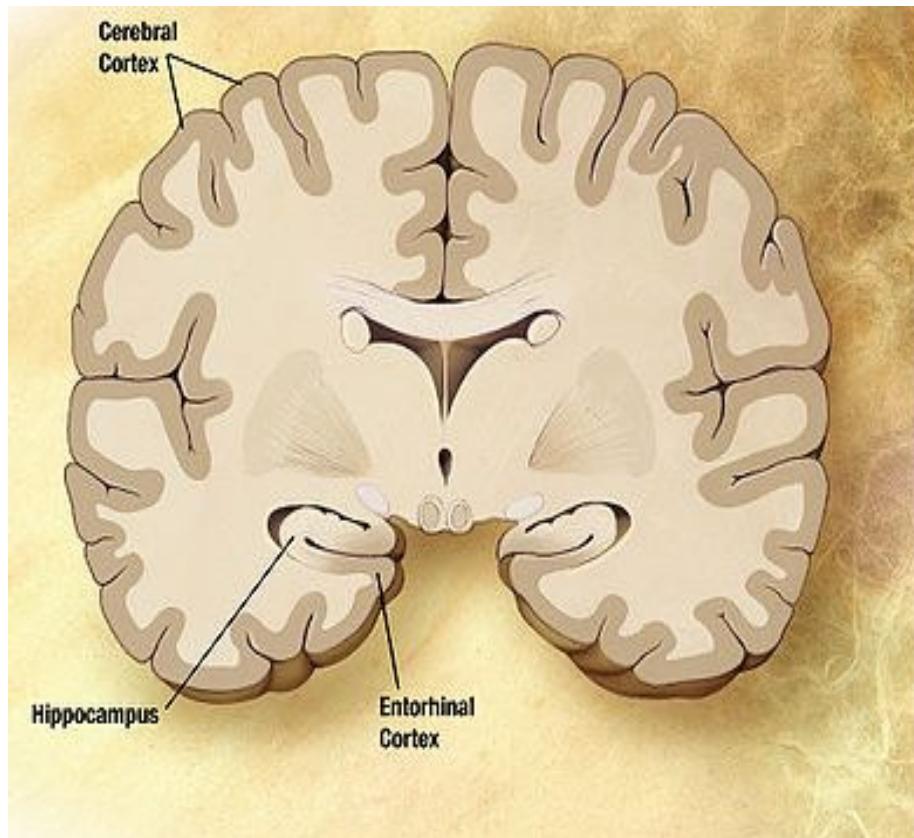
- most common form of senile dementia, 30-35 millions are affected (WHO) and in 2050 it's estimated that 106 millions may be affected
- age a risk factor. Above 80 years of age there is a 30% risk to develop the disease
- spontaneous (sAD) and familial forms (fAD, 5%)



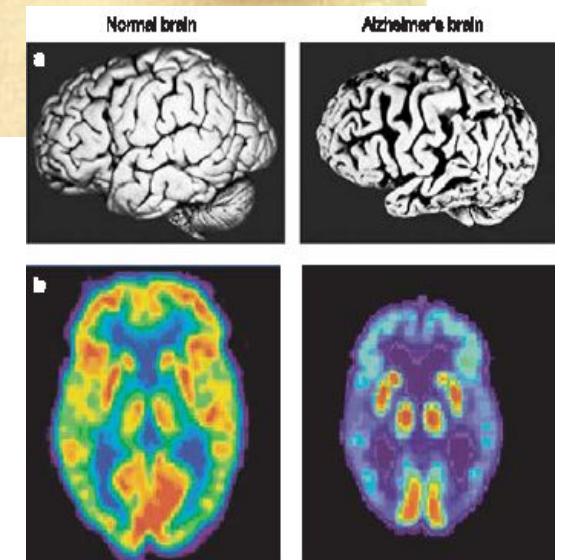
*Alzheimer*

Brookmeyer *et al.*, Alzheimer's and Dementia 3(3) 186 (2007)  
Dementia Fact sheet, WHO (2017)

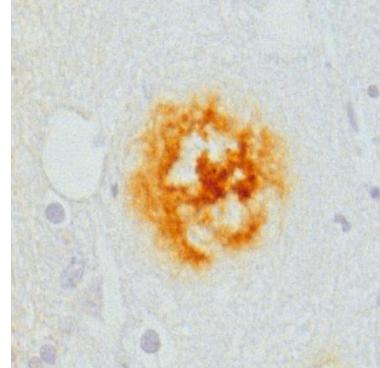
# Alzheimer's disease (AD)



In Alzheimer's disease synaptic loss and death of neurons occur both in cerebral cortex and in subcortical regions.

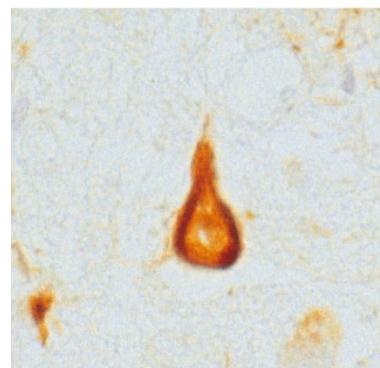


# Alzheimer's disease (AD)



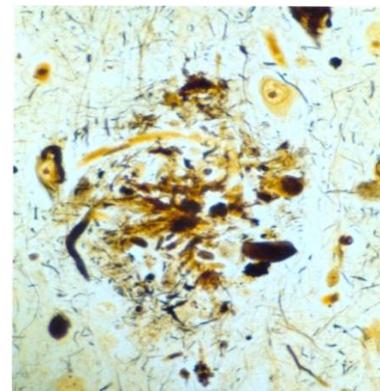
Senile plaque:

- $\beta$ -amyloid (A $\beta$ )
- ApoE



Neurofibrillary tangle:

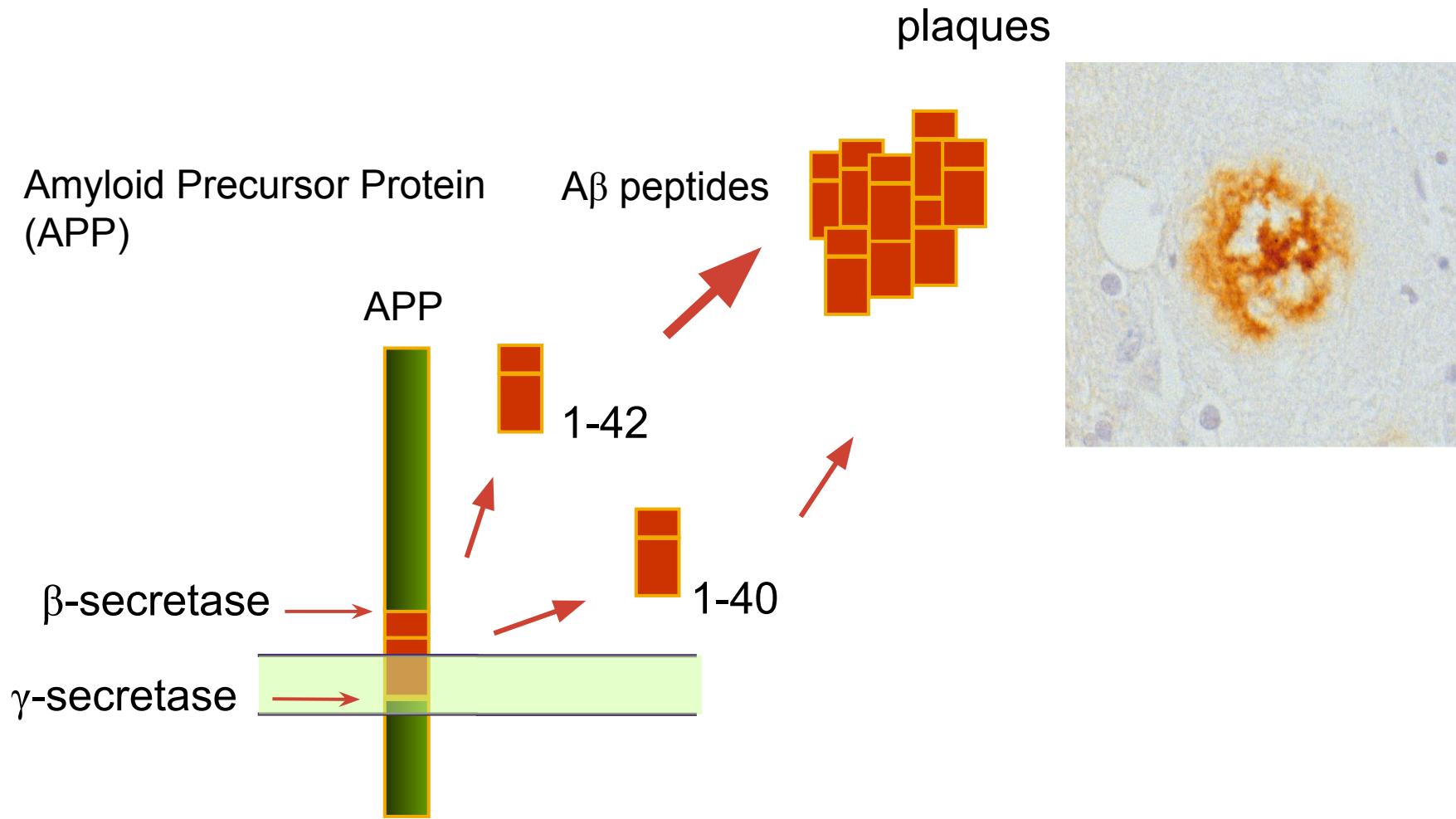
- tau



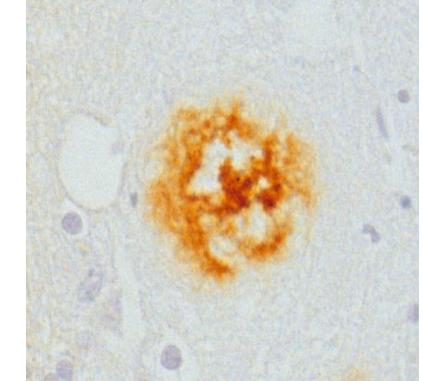
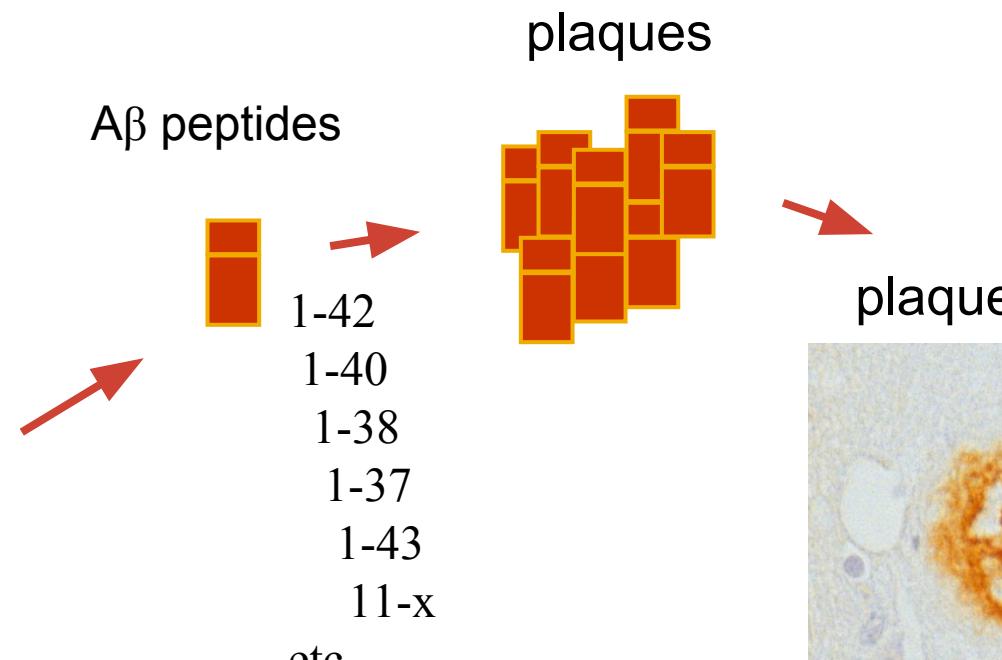
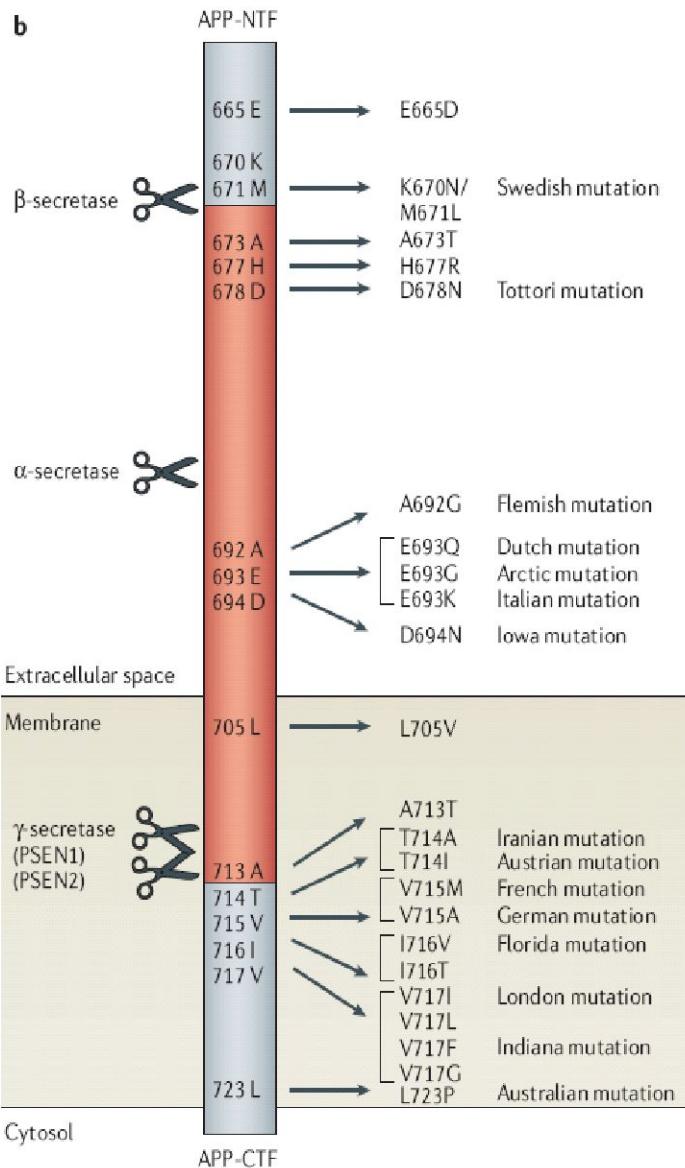
Neuritic plaque:

- A $\beta$
- tau
- swollen neurites

# What is A $\beta$ ?



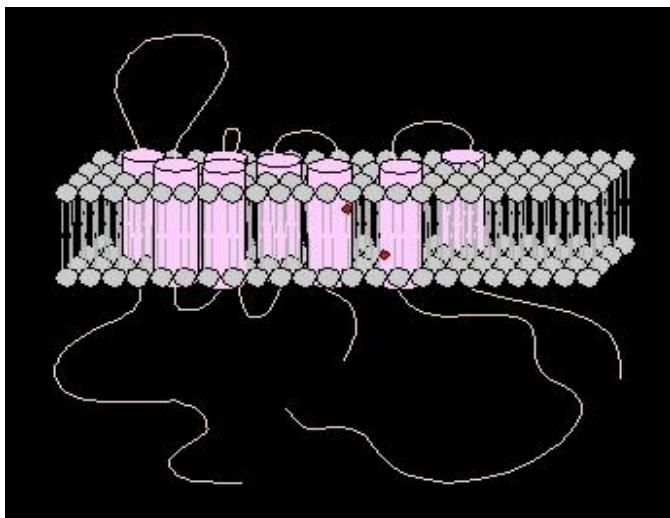
# FAD mutant APP results in increased or altered A $\beta$ production



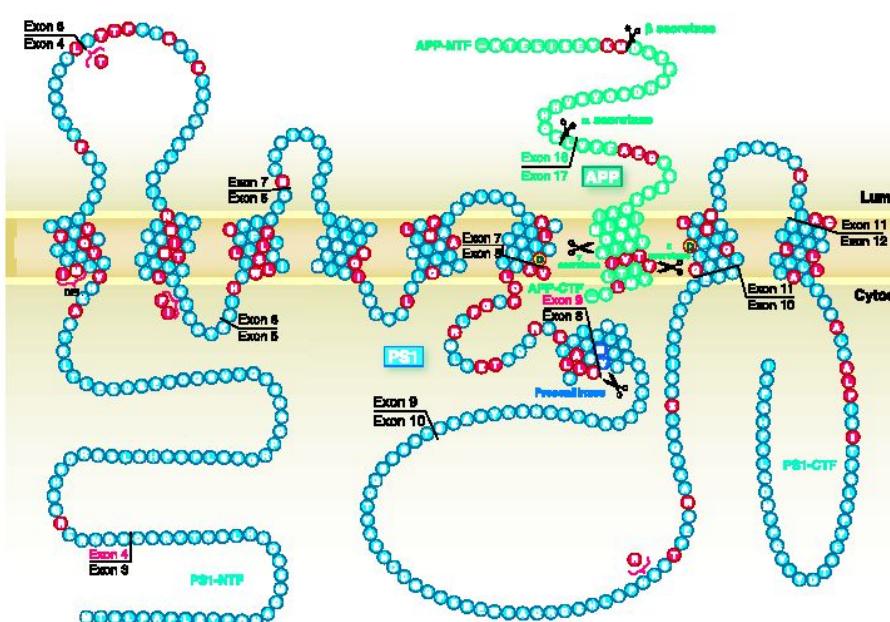
Van Dam & De Deyn, Nature Reviews Drug Discovery (2006)

# FAD mutant presenilin results in increased ratio of fibrillogenic A $\beta$

## Presenilin-1

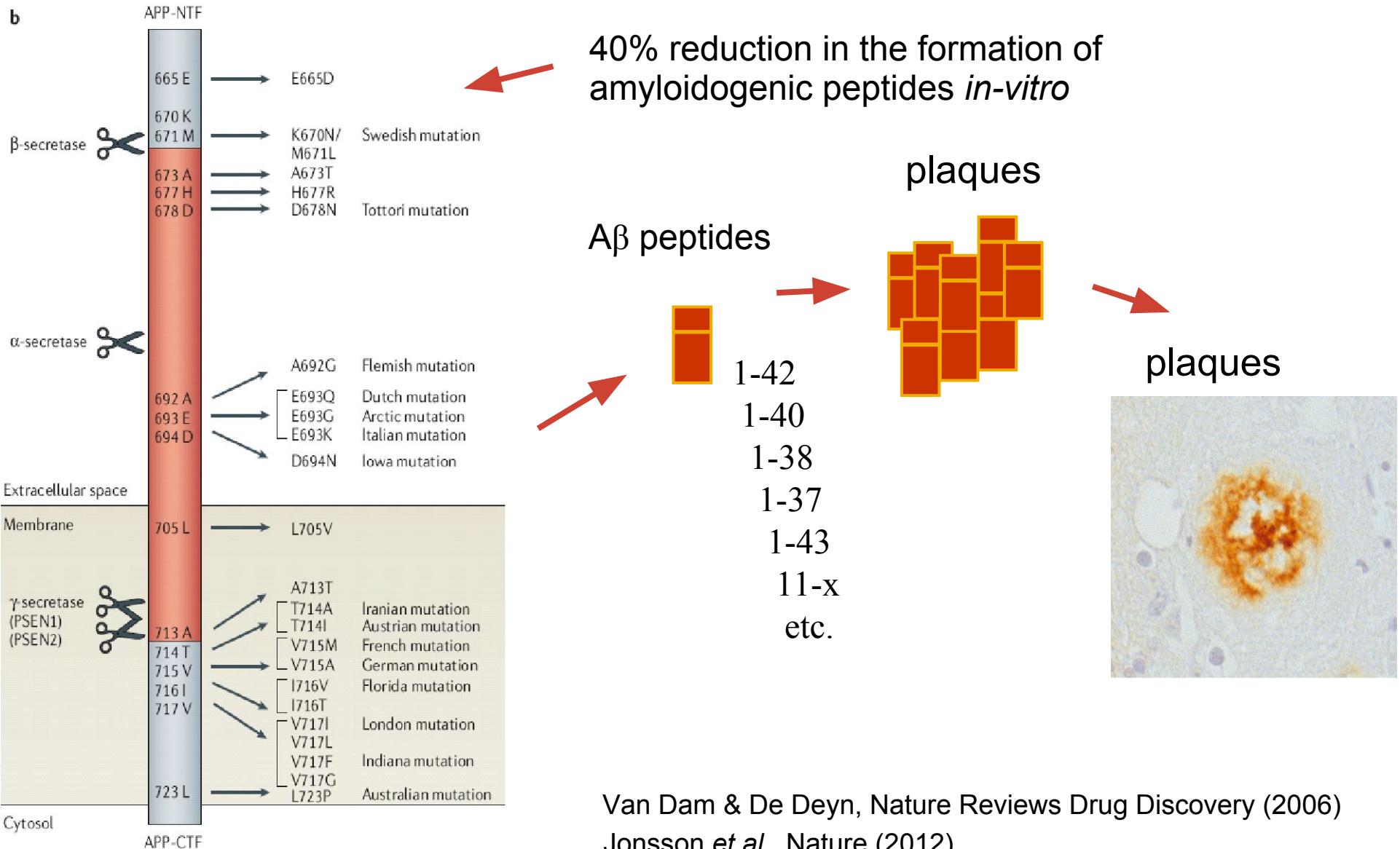


For details see: <https://www.alzforum.org/mutations/app>

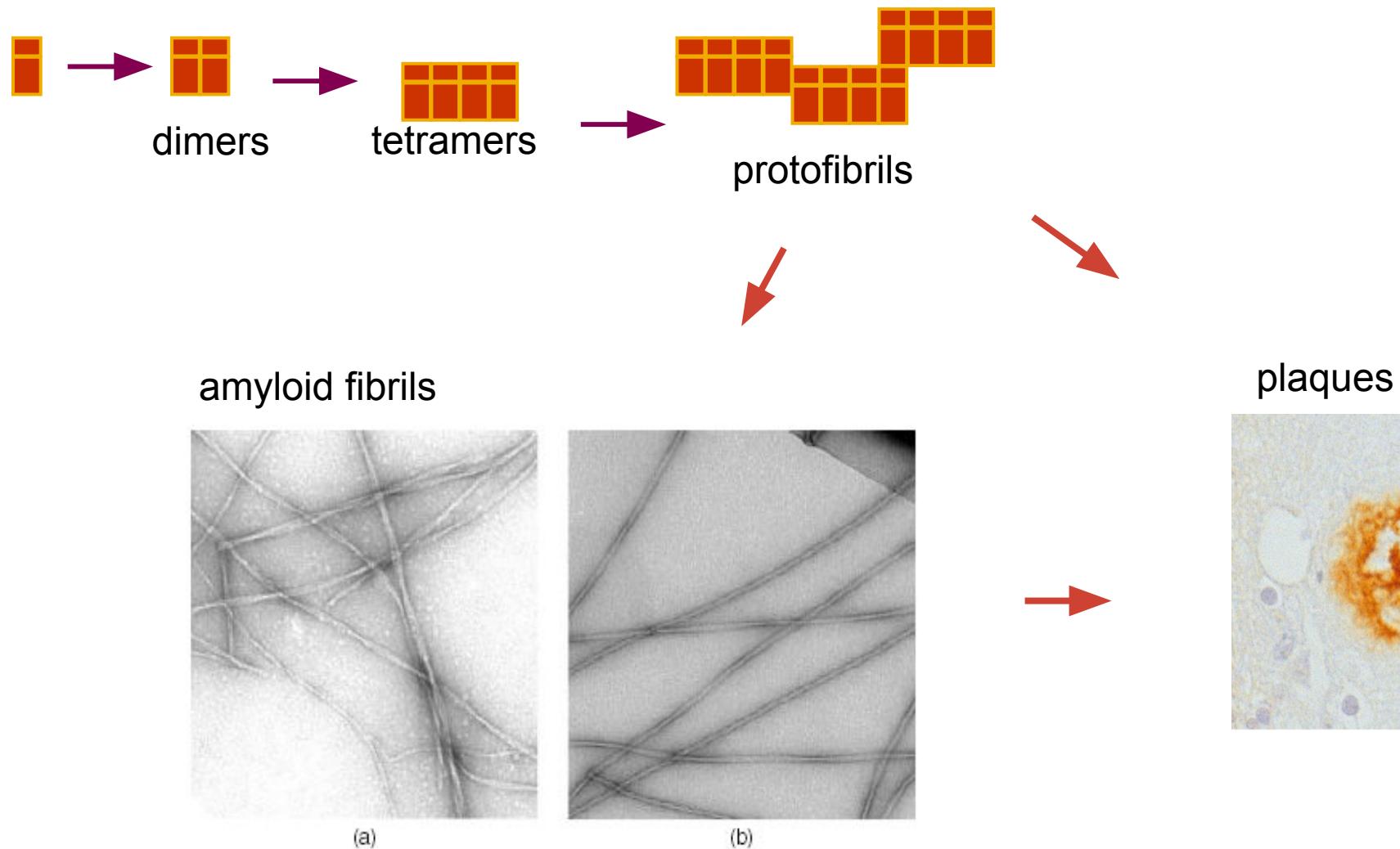


Mutations increase the ratio  
of fibrillogenic species

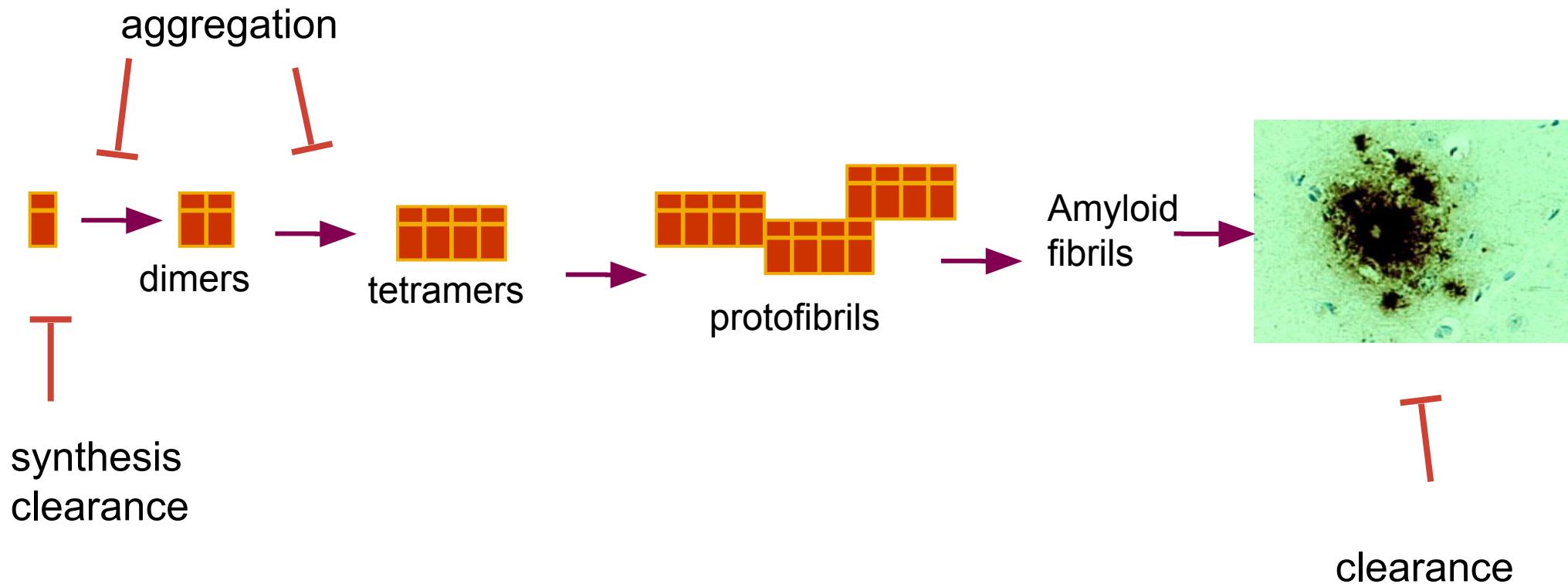
# A mutation in APP *protects* against AD and age-related cognitive decline



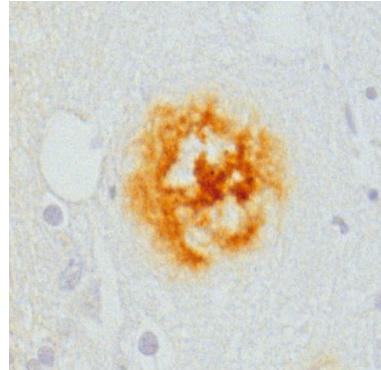
# What is the toxic species of A $\beta$ ?



# How can we interfere with A $\beta$ in AD ?

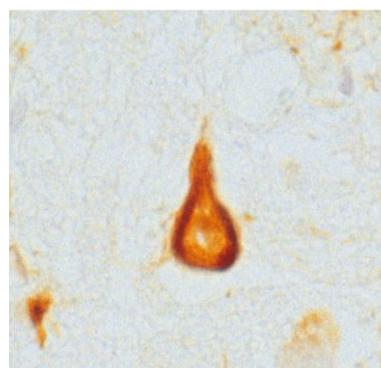


# Alzheimer's disease (AD)



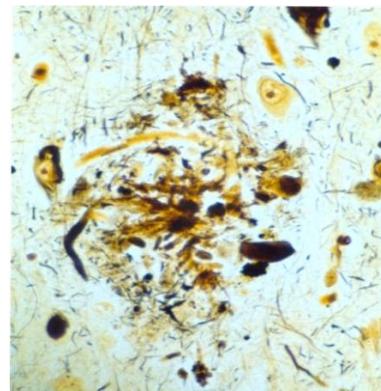
Senile plaque:

- $\beta$ -amyloid (A $\beta$ )
- ApoE



Neurofibrillary tangle:

- tau



Neuritic plaque:

- A $\beta$
- tau
- swollen neurites

# Neurofibrillary tangles, the role of tau

Götz & Ittner, Nature Rev. Neurosci. 9, 532 (2008)

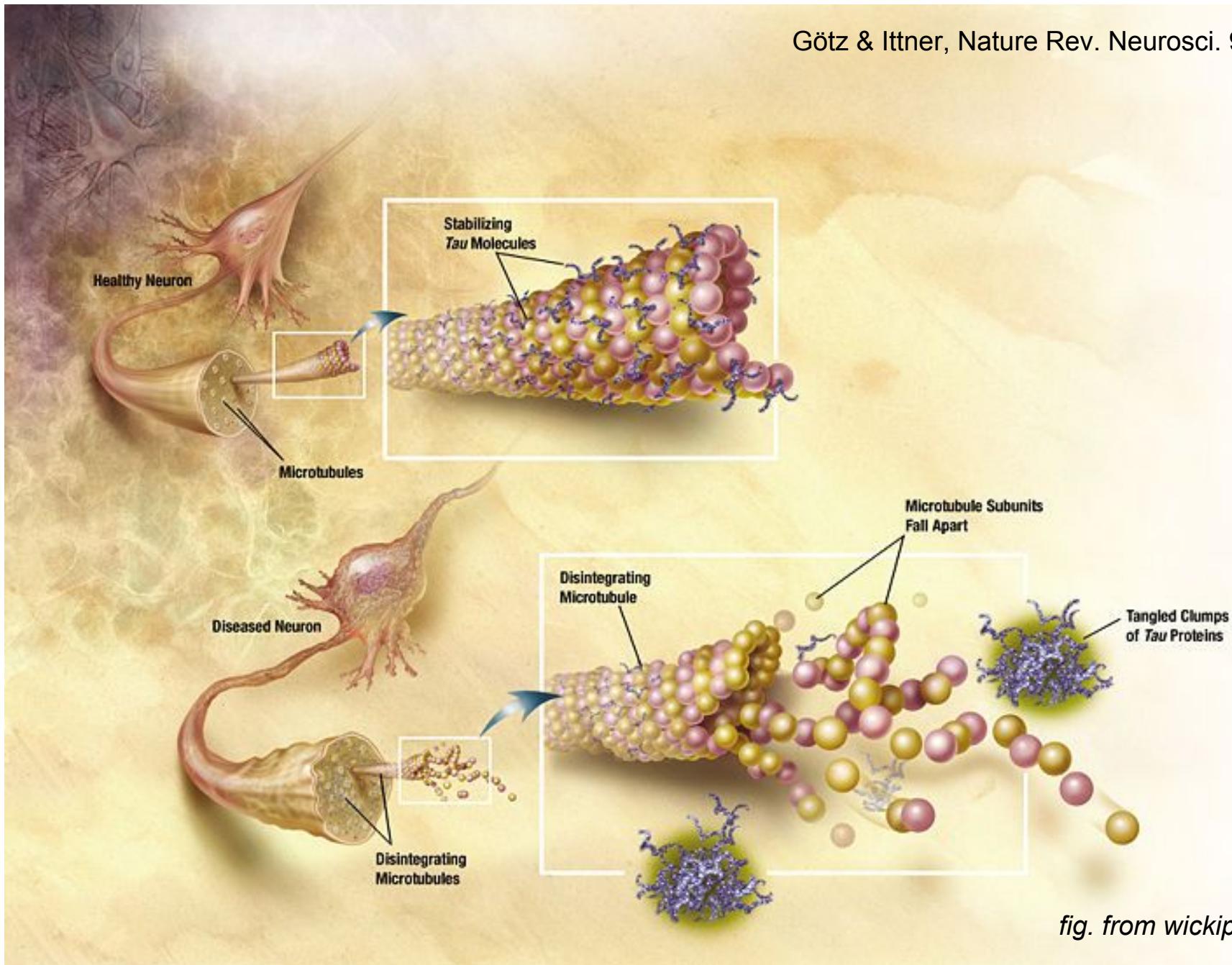
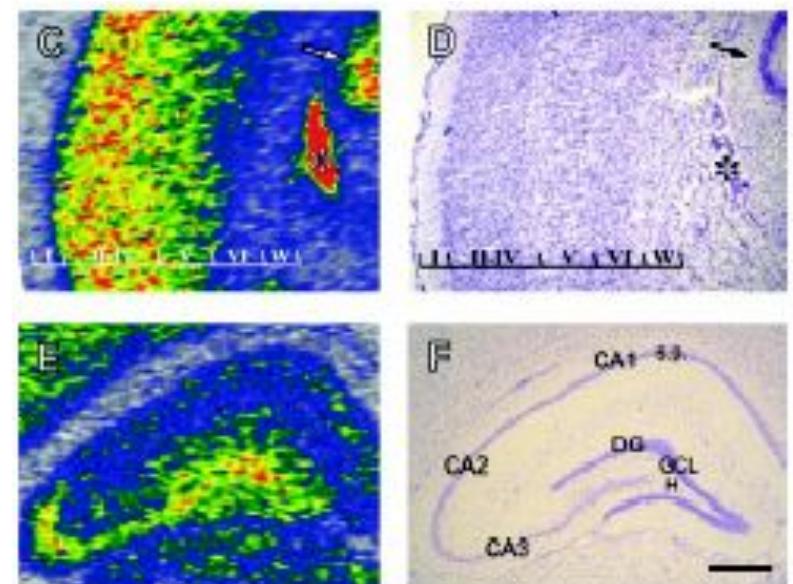


fig. from wikipedia.org

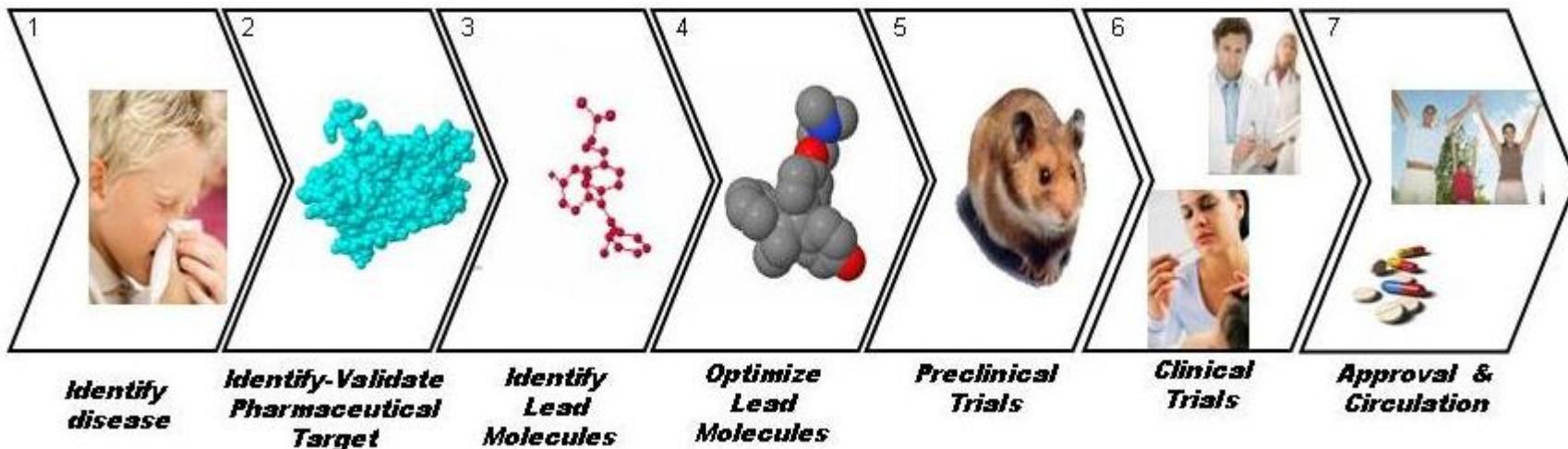
# Why are certain brain regions affected in particular?

Areas of the brain that display high synaptic plasticity and neurogenesis are more vulnerable

- The amyloid cascade hypothesis
- Axonal transport dysfunction hypotheses
- Apolipoprotein E and AD:  
Effects of ApoE on A $\beta$  metabolism suggest underlying mechanisms and potential treatments
- AD, PD, HD, ALS, FTLD, CJD and autoimmunity?
- Chronic stress hypotheses?



# The Drug Discovery Process



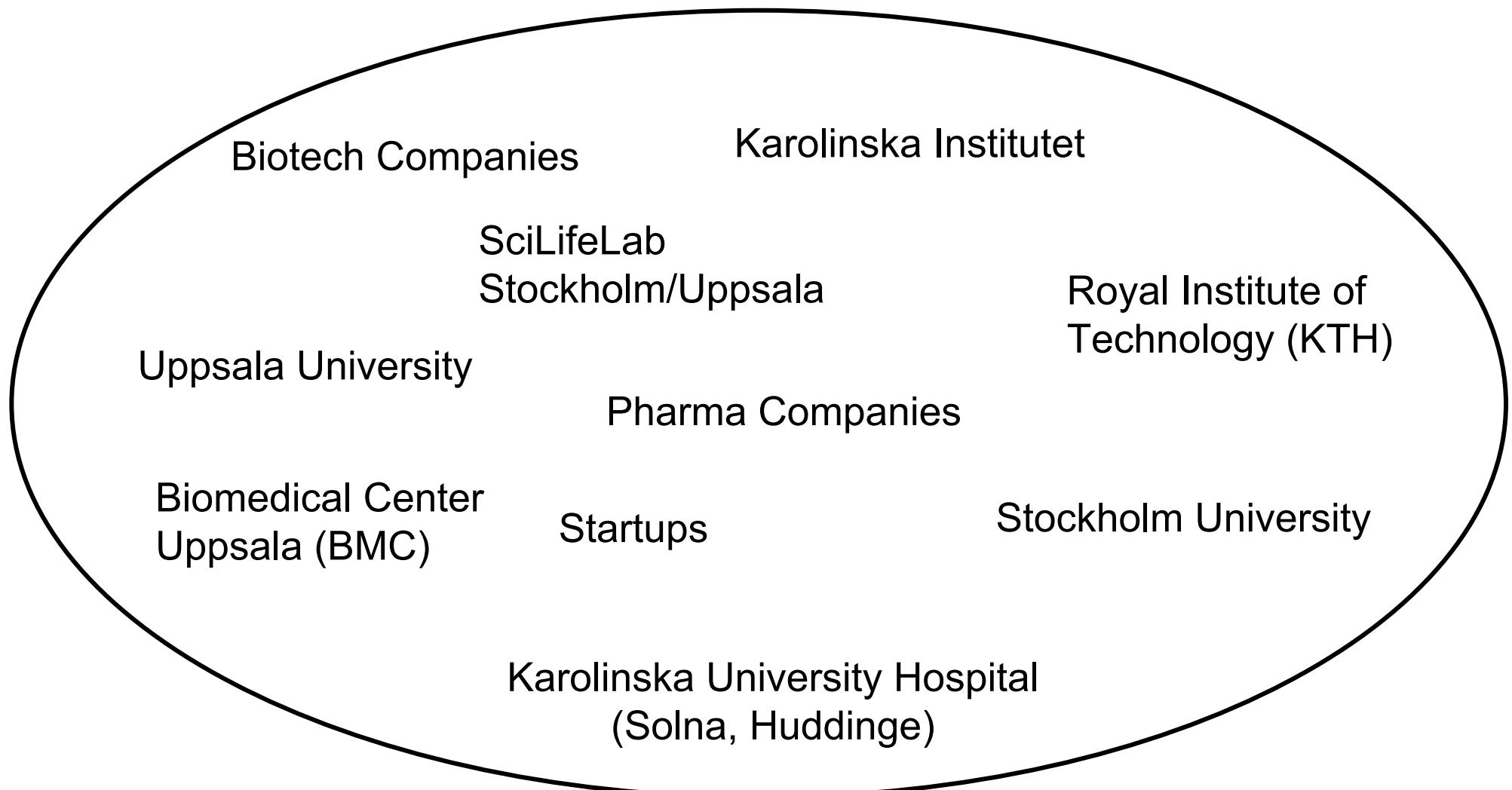
The process begins by focusing on areas of unmet medical need

We want to identify biological targets that have the potential to be starting points for pharmaceutical development

The challenges involve time and costs

# Infrastructure Greater Stockholm Area

What is our role ?



# The Drug Discovery Process

How to manage the challenges?

- Speculative research targets
- New target ideas

- Innovative improvements using existing targets and drugs
  - Higher potency
  - Higher selectivity
  - Less adverse effects

Risk

High

Moderate

# Challenges - Failures

## Right target / efficacy

- Target linkage to disease biology not fully established
- Preclinical models not available / not validated
- Clinical efficacy biomarkers not available / not validated

## Right tissue / right exposure

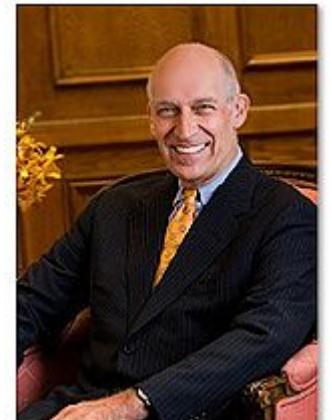
- Target/tissue hit not fully established
- Target engagement not fully established
- High PK/PD variability

## Right patient

## Right safety

*"Pharmacokinetics may be simply defined as what the body does to the drug, as opposed to pharmacodynamics which may be defined as what the drug does to the body. "*

*(ref: Leslie Z. Benet)*



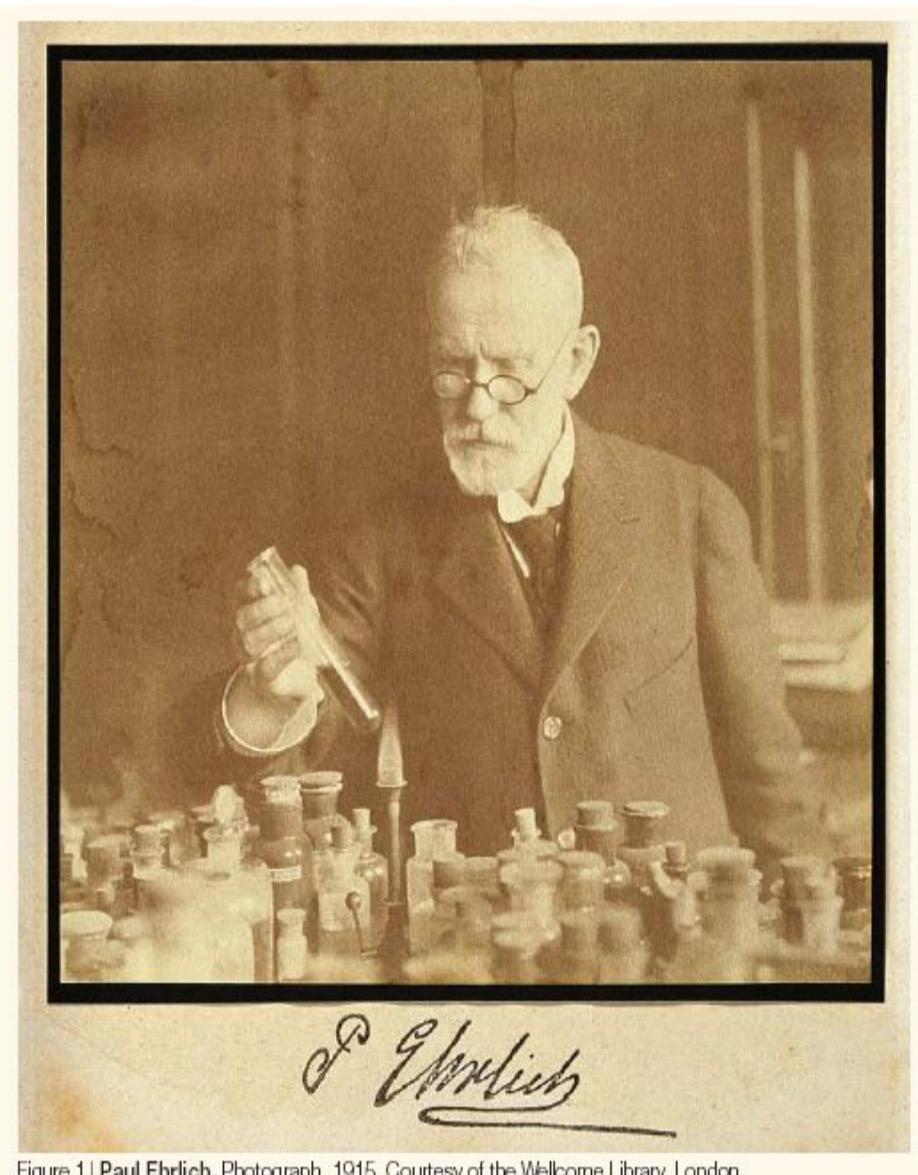
# The Drug Discovery Process

In the past:

identification of compounds

lead development

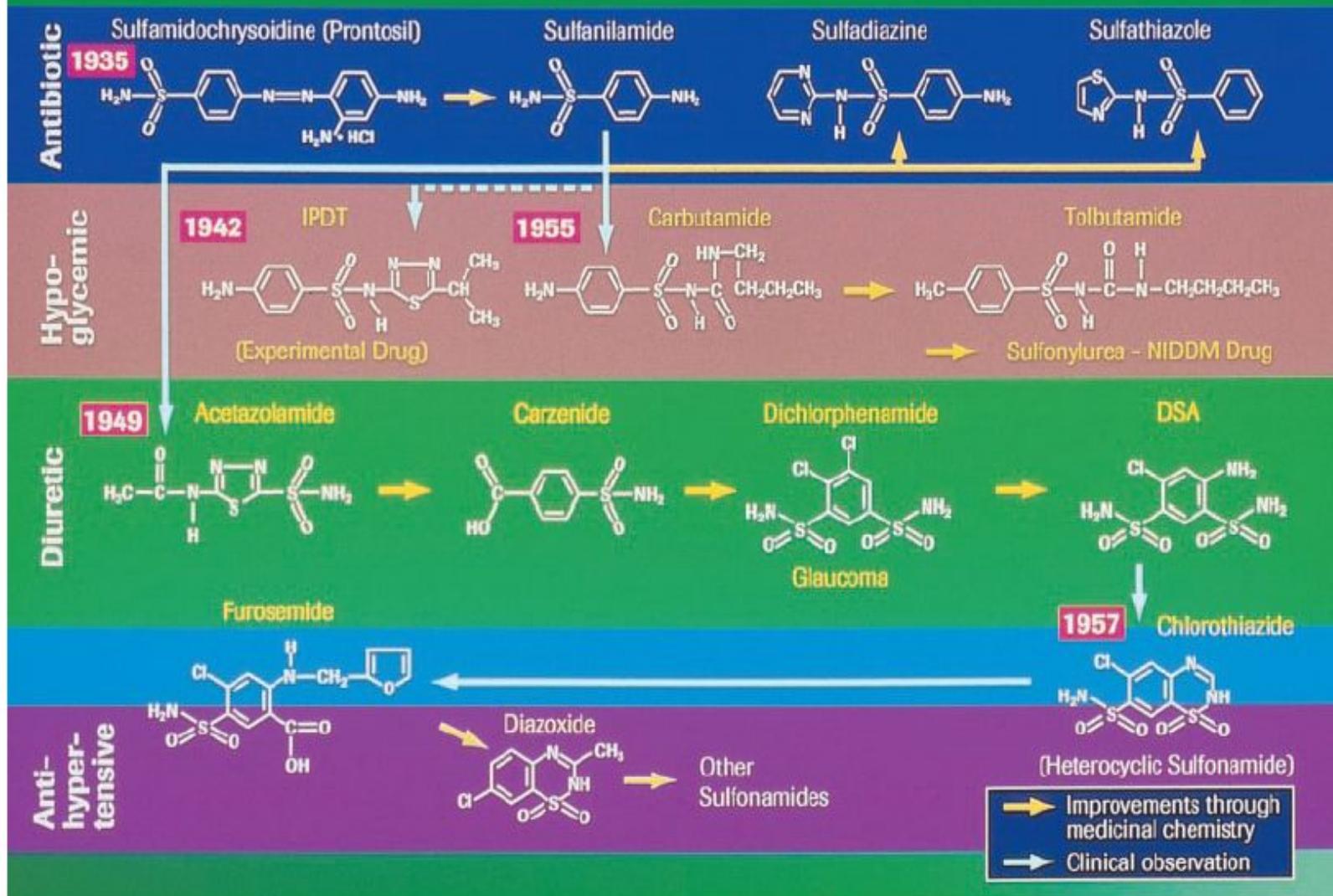
*in vitro / in vivo* disease models



Drews, Nat Rev Drug Discov. 9, 797 (2004)

Figure 1 | Paul Ehrlich. Photograph, 1915. Courtesy of the Wellcome Library, London.

## Sulfanilamides as Therapeutics



Drews, Science 287, 1960 (2000)

# The Drug Discovery Process

in the past...

identification of compounds

lead development

*in vitro / in vivo* disease models

later...

validation of drug targets

high throughput screens

lead development

*in vitro / in vivo* disease models



**articles**

# Initial sequencing and analysis of the human genome

**International Human Genome Sequencing Consortium\***

\*A partial list of authors appears on the opposite page. Affiliations are listed at the end of the paper.

The human genome holds an extraordinary trove of information about human development, physiology, medicine and evolution. Here we report the results of an international collaboration to produce and make freely available a draft sequence of the human genome. We also present an initial analysis of the data, describing some of the insights that can be gleaned from the sequence.

from: **Nature 409**, 860 (2001)

## The Sequence of the Human Genome

J. Craig Venter,<sup>1\*</sup> Mark D. Adams,<sup>1</sup> Eugene W. Myers,<sup>1</sup> Peter W. Li,<sup>1</sup> Richard J. Mural,<sup>1</sup> Granger G. Sutton,<sup>1</sup> Hamilton O. Smith,<sup>1</sup> Mark Yandell,<sup>1</sup> Cheryl A. Evans,<sup>1</sup> Robert A. Holt,<sup>1</sup> Jeannine D. Gocayne,<sup>1</sup> Peter Amanatides,<sup>1</sup> Richard M. Ballew,<sup>1</sup> Daniel H. Huson,<sup>1</sup> Jennifer Russo Wortman,<sup>1</sup> Qing Zhang,<sup>1</sup> Chinnappa D. Kodira,<sup>1</sup> Xiangqun H. Zheng,<sup>1</sup> Lin Chen,<sup>1</sup> Marian Skupski,<sup>1</sup> Gangadharan Subramanian,<sup>1</sup> Paul D. Thomas,<sup>1</sup> Jinghui Zhang,<sup>1</sup> George L. Gabor Miklos,<sup>2</sup> Catherine Nelson,<sup>3</sup> Samuel Broder,<sup>1</sup> Andrew G. Clark,<sup>4</sup> Joe Nadeau,<sup>5</sup> Victor A. McKusick,<sup>6</sup> Norton Zinder,<sup>7</sup> Arnold J. Levine,<sup>7</sup> Richard J. Roberts,<sup>8</sup> Mel Simon,<sup>9</sup>



from: **Science 291**, 1304 (2001)

# Finishing the euchromatic sequence of the human genome

**International Human Genome Sequencing Consortium\***

*\* A list of authors and their affiliations appears in the Supplementary Information*

The sequence of the human genome encodes the genetic instructions for human physiology, as well as rich information about human evolution. In 2001, the International Human Genome Sequencing Consortium reported a draft sequence of the euchromatic portion of the human genome. Since then, the international collaboration has worked to convert this draft into a genome sequence with high accuracy and nearly complete coverage. Here, we report the result of this finishing process. The current genome sequence (Build 35) contains 2.85 billion nucleotides interrupted by only 341 gaps. It covers ~99% of the euchromatic genome and is accurate to an error rate of ~1 event per 100,000 bases. Many of the remaining euchromatic gaps are associated with segmental duplications and will require focused work with new methods. The near-complete sequence, the first for a vertebrate, greatly improves the precision of biological analyses of the human genome including studies of gene number, birth and death. Notably, the human genome seems to encode only 20,000–25,000 protein-coding genes. The genome sequence reported here should serve as a firm foundation for biomedical research in the decades ahead.

*from: Nature 431, 931(2004)*

# PERSPECTIVES

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## OPINION

### How many drug targets are there?

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*John P. Overington, Bissan Al-Lazikani and Andrew L. Hopkins*

**Abstract** | For the past decade, the number of molecular targets for approved drugs has been debated. Here, we reconcile apparently contradictory previous reports into a comprehensive survey, and propose a consensus number of current drug targets for all classes of approved therapeutic drugs. One striking feature is the relatively constant historical rate of target innovation (the rate at which drugs against new targets are launched); however, the rate of developing drugs against new families is significantly lower. The recent approval of drugs that target protein kinases highlights two additional trends: an emerging realization of the importance of polypharmacology, and also the power of a gene-family-led approach in generating novel and important therapies.

*from: Nature Reviews Drug Discovery 5, 993 (2006)*

Table 1 | Molecular targets of FDA-approved drugs

Drug target class	Targets			Drugs		
	Total targets	Small-molecule drug targets	Biologic drug targets	Total drugs	Small molecules	Biologics
Human protein	667	549	146	1,194	999	195
Pathogen protein	189	184	7	220	215	5
Other human biomolecules	28	9	22	98	63	35
Other pathogen biomolecules	9	7	4	79	71	8

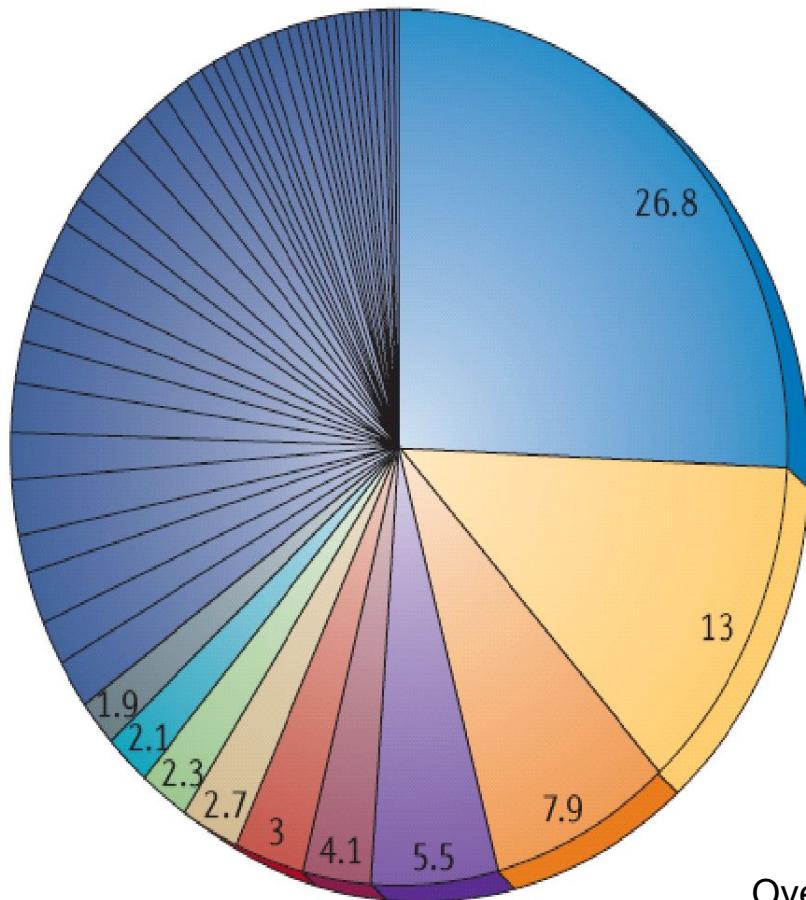
The list also includes antimalarial drugs approved elsewhere in the world.

Santos *et al.*, Nature Reviews Drug Discovery **16**, 19 (2017)

Number of targets?

- Conservative estimates approx. 1000 (Overington *et al.*)

# Current Drug Targets / Target Classes



- Rhodopsin-like GPCRs
- Nuclear receptors
- Ligand-gated ion channels
- Voltage-gated ion channels
- Penicillin-binding protein
- Myeloperoxidase-like
- Sodium: neurotransmitter symporter family
- Type II DNA topoisomerase
- Fibronectin type III
- Cytochrome P450

**Figure 1 | Gene-family distribution of current drugs per drug substance.** The family share as a percentage of all FDA-approved drugs is displayed for the top ten families.

Overington *et al.*, Nature Reviews Drug Discovery 5, 993 (2006)

# The Drug Discovery Process

... and more recent...

identification of compounds

lead development

*in vitro / in vivo* disease models

later...

validation of drug targets

high throughput screens

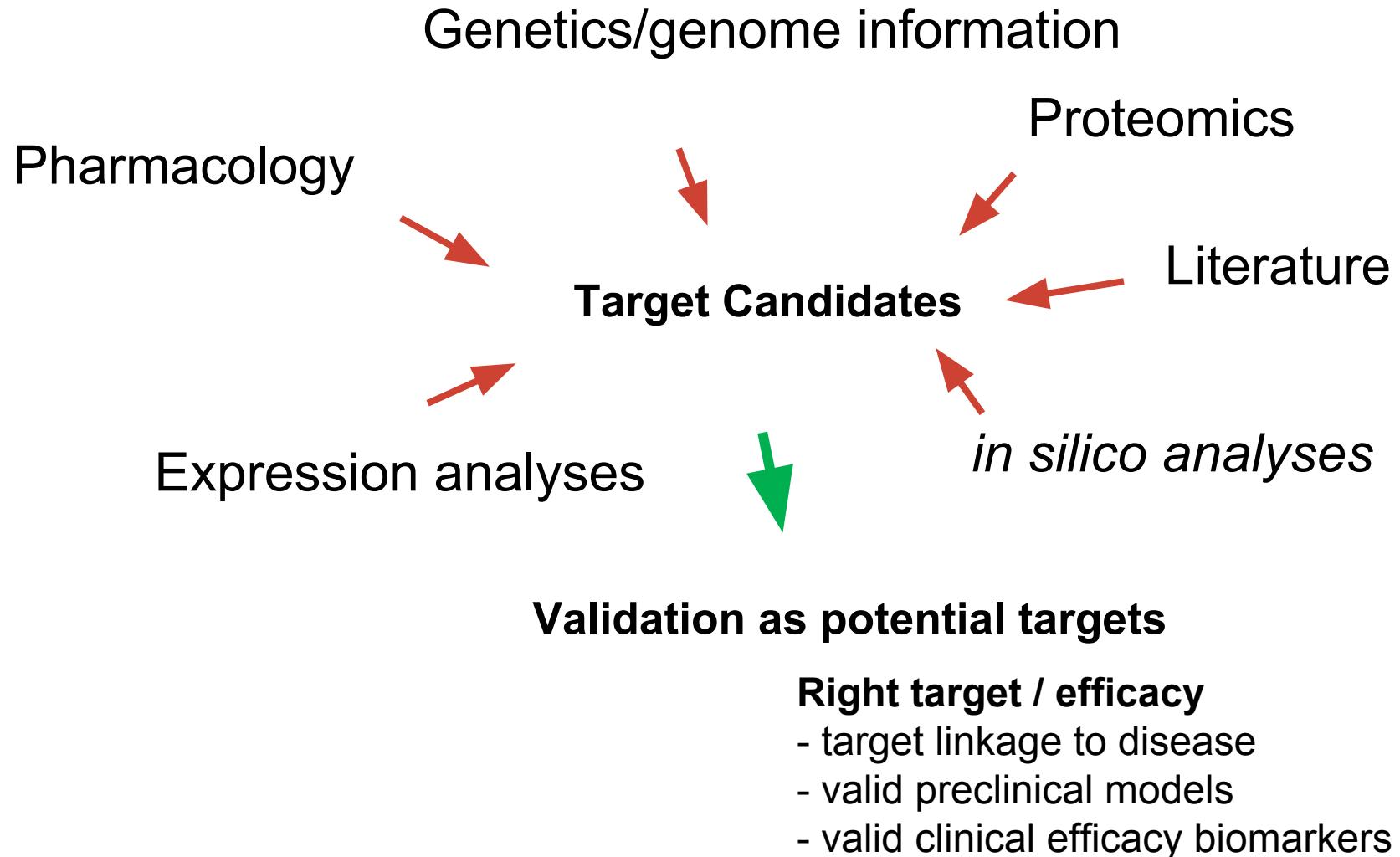
lead development

*in vitro / in vivo* disease models



# The Drug Discovery Process

Some different ways to identify and validate possible targets...



# FUNCTIONAL GENOMICS TO NEW DRUG TARGETS

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*Richard Kramer and Dalia Cohen*

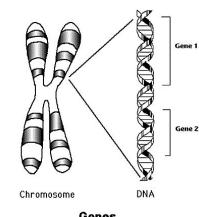
**Abstract** | The completion of the sequencing of the human genome, and those of other organisms, is expected to lead to many potential new drug targets in various diseases, and it is predicted that novel therapeutic agents will be developed against such targets. The role of functional genomics in modern drug discovery is to prioritize these targets and to translate that knowledge into rational and reliable drug discovery. Here, we describe the field of functional genomics and review approaches that have been applied to drug discovery, including RNA profiling, proteomics, antisense and RNA interference, model organisms and high-throughput, genome-wide overexpression or knockdowns, and outline the future directions that are likely to yield new drug targets from genomics.

*from:* Nature Reviews Drug Discovery **3**, 965 (2004)

# Target validation



Literature study



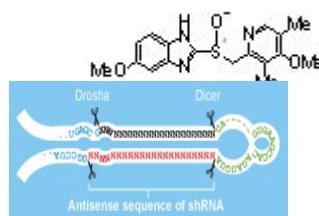
Genetic association to disease



Expression association to disease



Disease models



Compounds



# Literature study

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PubMed secretace

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Clinical Trial  
Review  
Customize ...

Text availability  
Abstract  
Free full text  
Full text

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[Indirect immobilized Jagged1 suppresses cell cycle progression and induces odonto/osteogenic differentiation in human dental pulp cells](#)

1. [Jagged1](#)

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Sim

Google Scholar

Artiklar

secretace



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[Def](#)

2. [Flu](#)

När som helst

Ch  
CE Sedan 2018

Clin Sedan 2017

PMI Sedan 2014

Sim Anpassat intervall ...

[Eng](#)

3. [Act](#) Sortera efter relevans

Wa Sortera efter datum

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inkludera patent

inkludera citat

Skapa alarm

[HTML] [Alzheimer's-causing mutations shift A \$\beta\$  length by destabilizing  \$\gamma\$ -secretase-A \$\beta\$ n interactions](#)

M Szaruga, B Munteanu, S Lismont, S Veugelen... - *Cell*, 2017 - Elsevier

Summary Alzheimer's disease (AD)-linked mutations in Presenilins (PSEN) and the amyloid precursor protein (APP) lead to production of longer amyloidogenic A $\beta$  peptides. The shift in A $\beta$  length is fundamental to the disease; however, the underlying mechanism remains ...

Citerat av 21 Relaterade artiklar Alla 8 versionerna

**$\gamma$ -Secretase Inhibition Lowers Plasma Triglyceride-Rich Lipoproteins by Stabilizing the LDL Receptor**

KJ Kim, IJ Goldberg, MJ Graham, M Sundaram... - *Cell metabolism*, 2018 - Elsevier

Excess plasma triglycerides (TGs) are a key component of obesity-induced metabolic syndrome. We have shown that  $\gamma$ -secretase inhibitor (GSI) treatment improves glucose tolerance due to inhibition of hepatic Notch signaling but found additional Notch ...

Citerat av 1 Relaterade artiklar Alla 5 versionerna

# Literature studies, future directions, ctd...

Concepts found in this article

[What is this?](#)

Plasma A $\beta$  Level   **Plasma A $\beta$ 42 Level**   CSF A $\beta$ 42 Level   Total A $\beta$  Level   Ibuprofen Treatment   App Transgenic Mouse   CSF Sample   A $\beta$ 42 Ratio  
CHO Cell   A $\beta$ 42 Peptide   Conditioned Medium   Ibuprofen Group    $\gamma$ -secretase Activity

Related articles containing similar concepts (345 articles)

[Elevation in Plasma Abeta42 in Geriatric Depression: A Pilot Study](#)

Pomara, N. - Doraiswamy, P., et al. in *Neurochemical Research* (2006)

[Increased plasma amyloid- \$\beta\$ 42 protein in sporadic inclusion body myositis](#)

Abdo, F. - Mierlo, T., et al. in *Acta Neuropathologica* (2009)

[Maximizing the Potential of Plasma Amyloid-Beta as a Diagnostic Biomarker for Alzheimer's Disease](#)

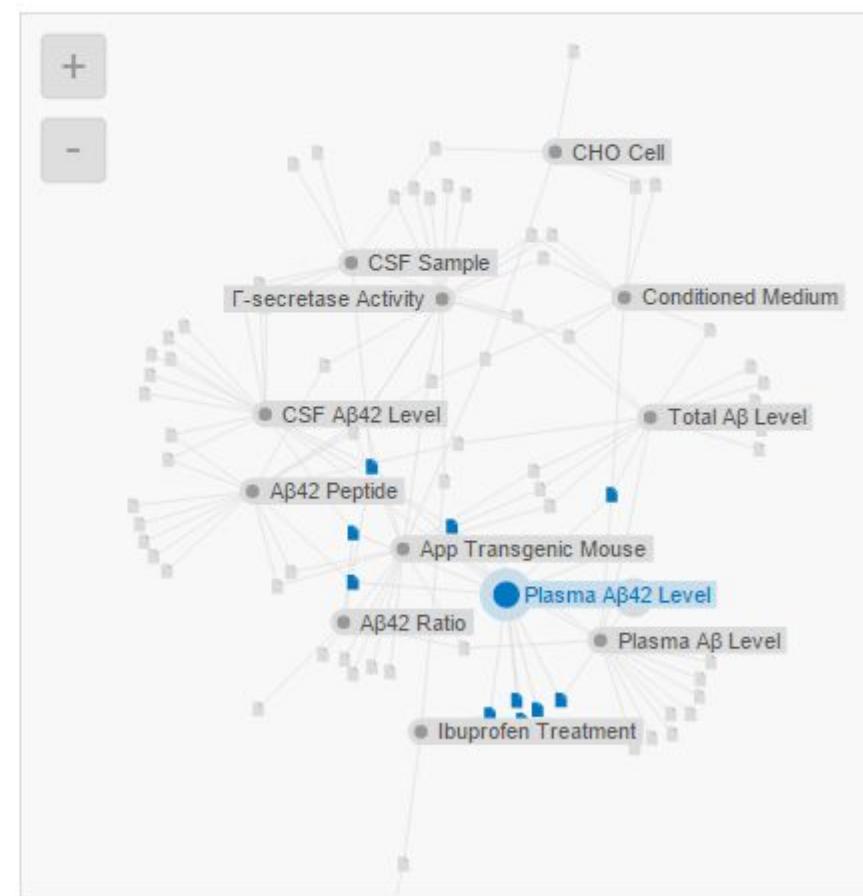
Oh, S. - Troncoso, J., et al. in *NeuroMolecular Medicine* (2008)

[Has inhibition of A \$\beta\$  production adequately been tested as therapeutic approach in mild AD? A model-based meta-analysis of  \$\gamma\$ -secretase inhibitor data](#)

Niva, C., et al. in *European Journal of Clinical Pharmacology* (2013)

[Efficacy of SPI-1865, a novel gamma-secretase modulator, in multiple rodent models](#)

Loureiro, M. - Dumin, A., et al. in *Alzheimer's Research & Therapy* (2013)



# Literature studies, future directions

## Literature text mining

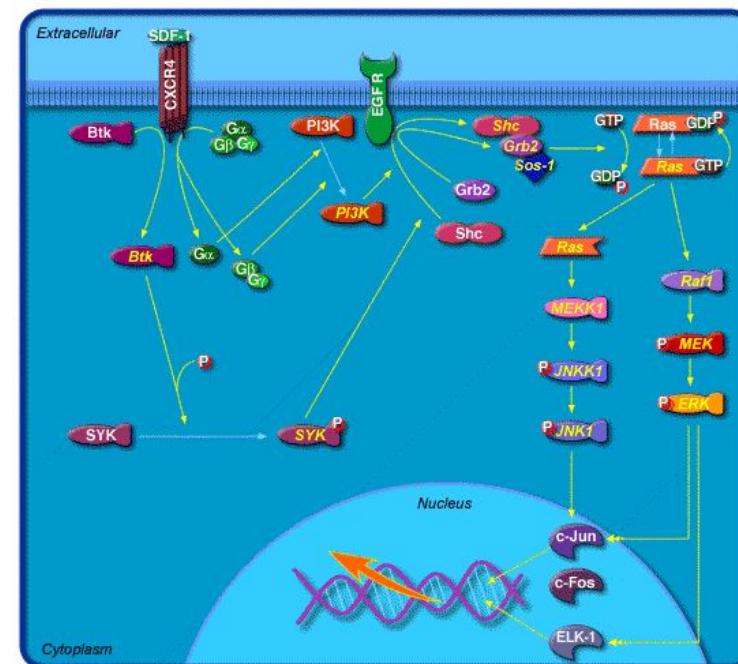
Establish relationship between candidate genes and disease pathways in an automated way

## Pathway mapping:

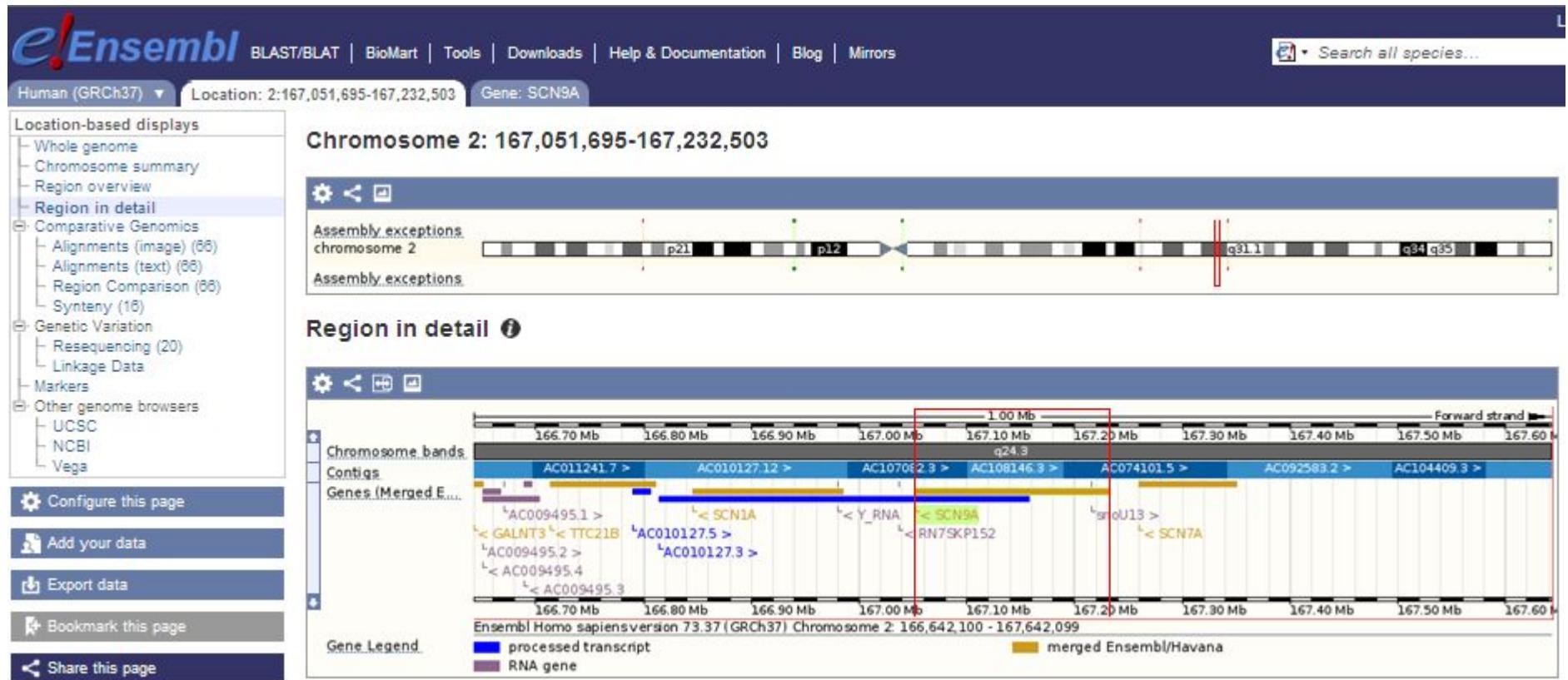
- Metabolic pathways
- Signalling pathways
- Gene regulation pathways
- Protein-protein interactions

## Generated from:

- literature data
- "omics" data



# Genome Informatics



- Use genome information for validation of existing targets (SNPs, splices, haplotypes)
- “All” genes identified, but few properly characterized
- Identify proteins with important target domains

# Protein domain databases

*for example:*

NCBI Concerved  
Domain Database

Domains can be described  
and used to:



- identify the putative function of a protein sequence
- identify the amino acids in a protein sequence that are putatively involved in functions such as binding or catalysis...
- find other proteins with similar domain architecture



## Target validation Literature study

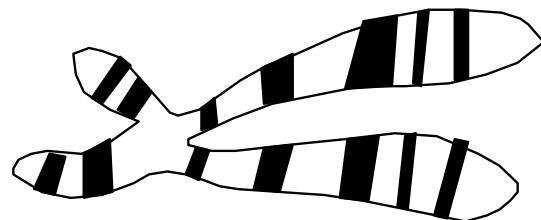
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- Traditional approach
- Very successful
- Include pathway mapping

-

- Need to verify data *in-house*
- Competition with other pharma

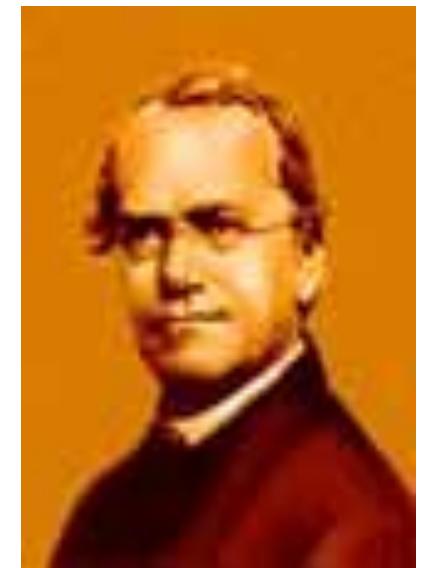
# Genetic association to disease



genome wide scans



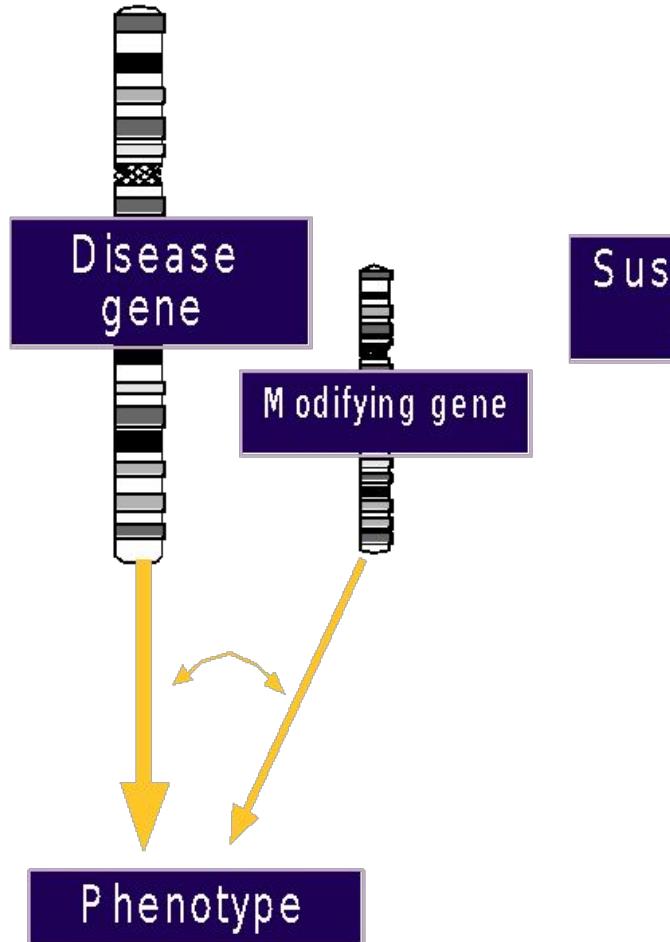
genetic markers:linkage



# Genetic Traits

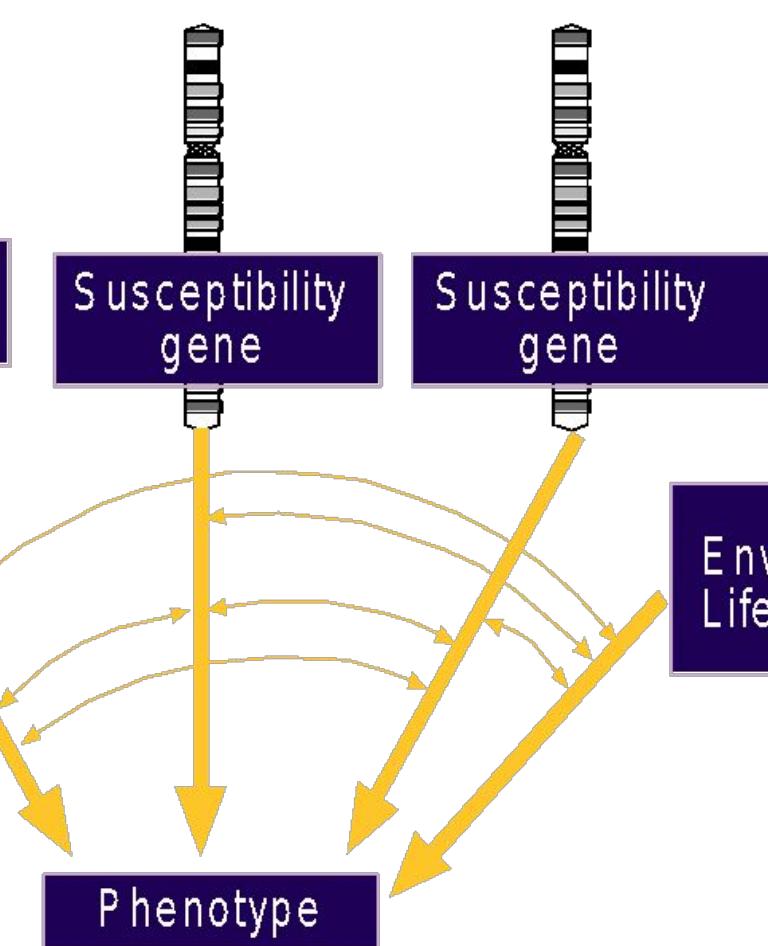
Simplex or monogenic

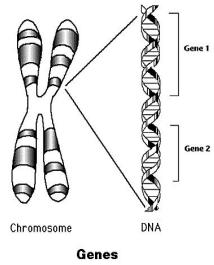
Monogenic



Complex or multifactorial

Polygenic





# Target validation

## Genetic association to disease

+

Direct link between target and disease

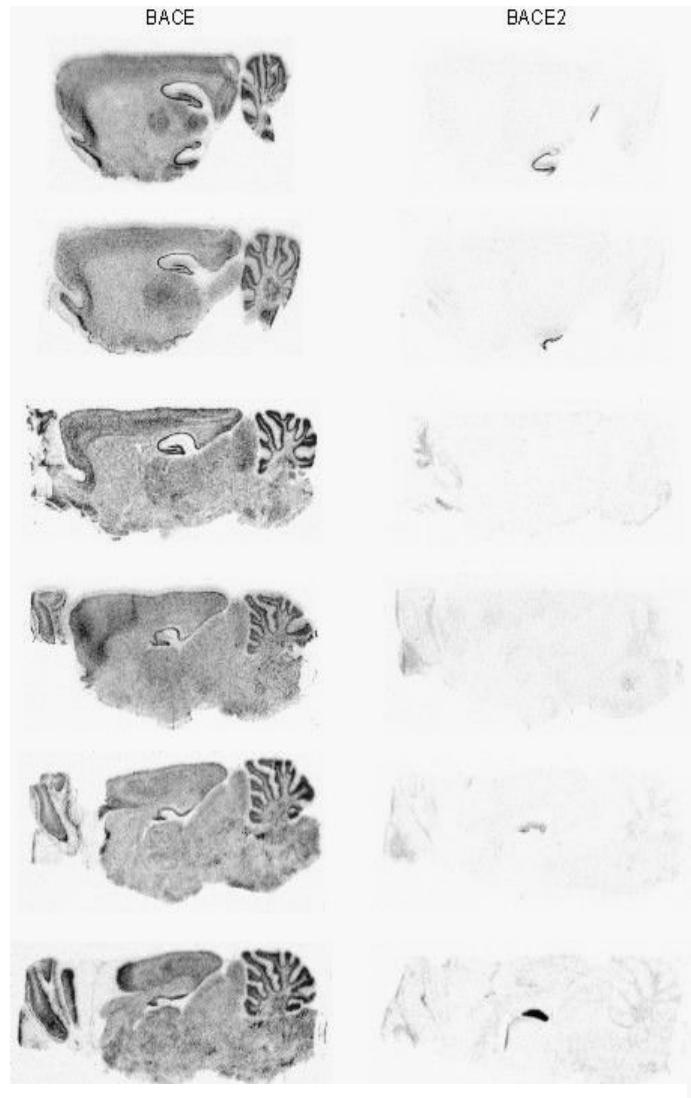
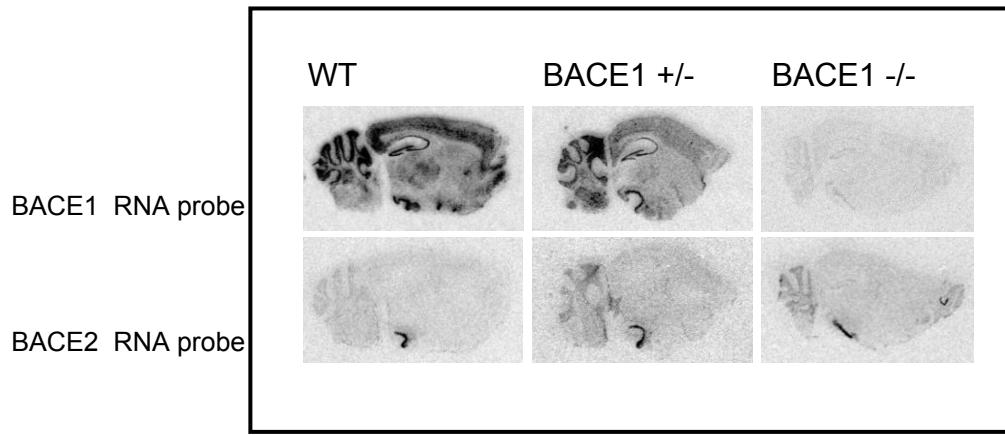
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Target not always druggable  
Needs well defined clinical material with  
uniformed diagnosis

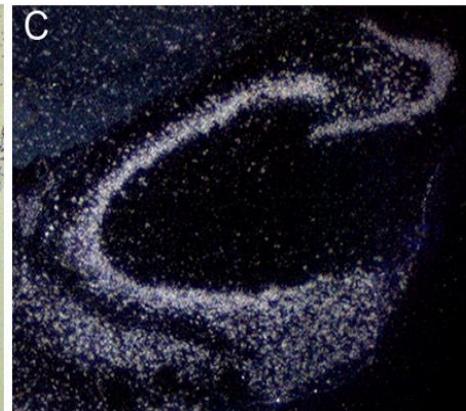
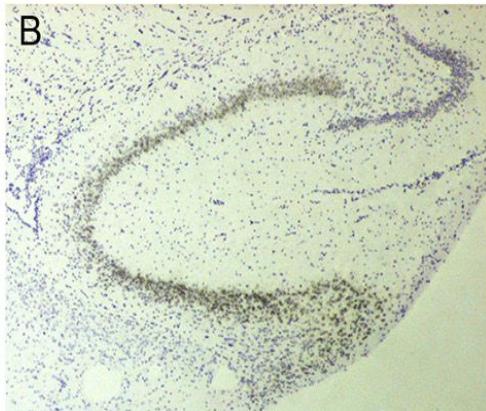
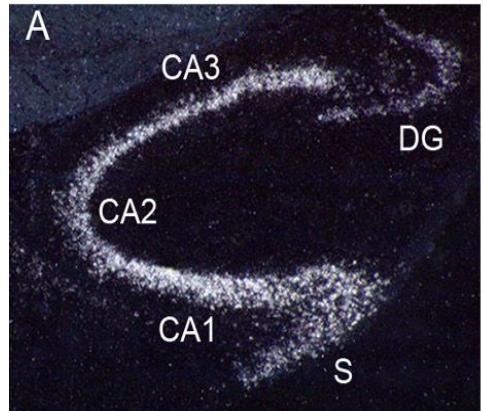


# Expression association to disease

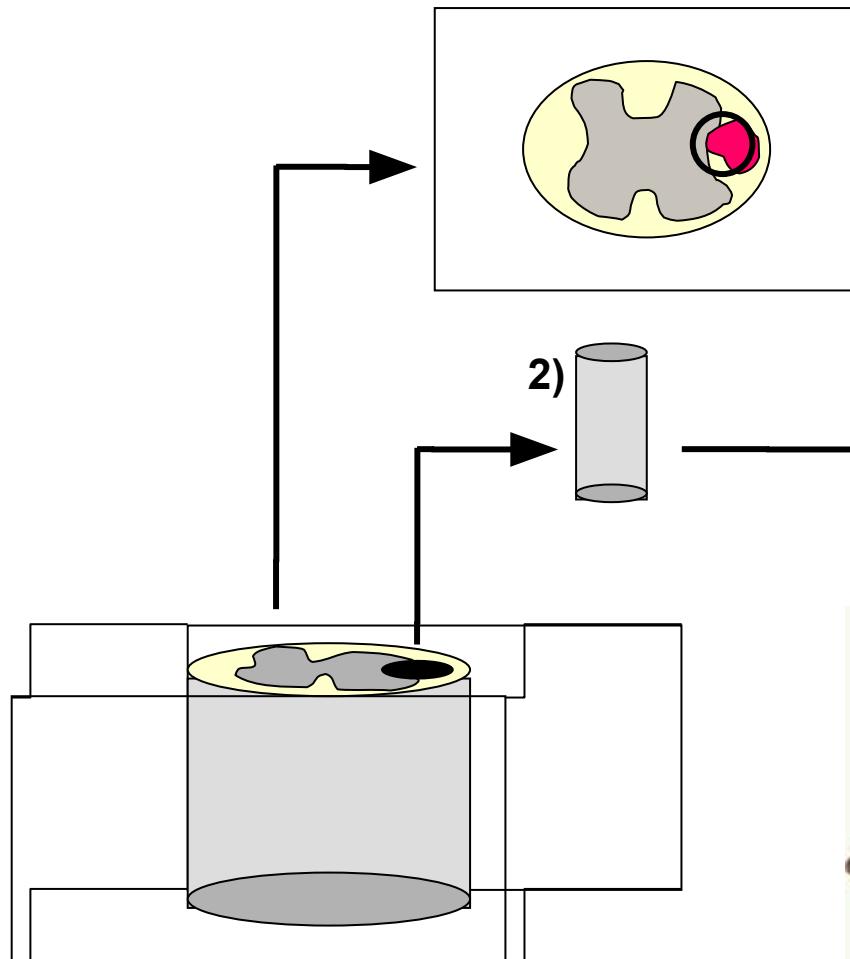
*In-situ hybridization (ISH) to localize  
BACE1 och BACE2 in rat brain*



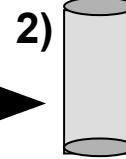
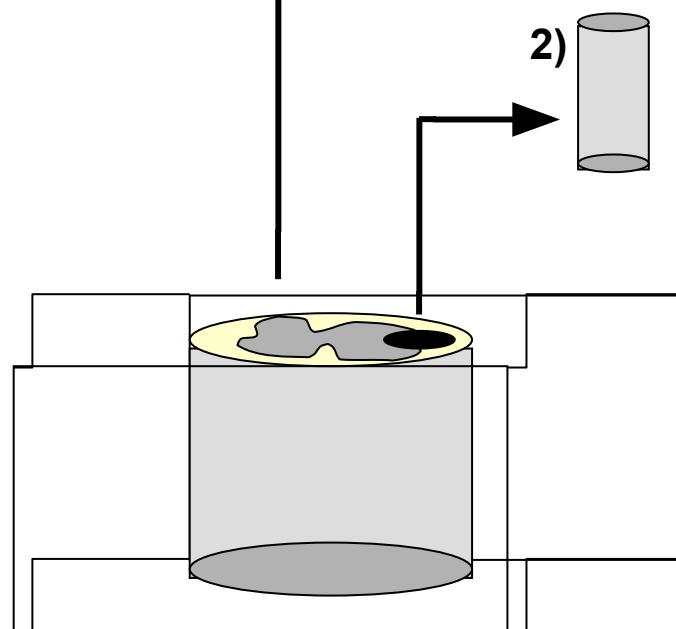
**BACE2**



# Tissue microarray assembly

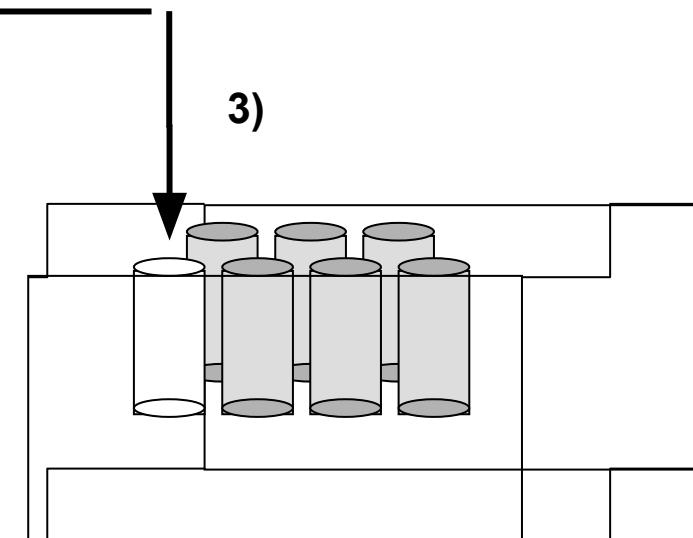


1) Section from donor block to define region of interest



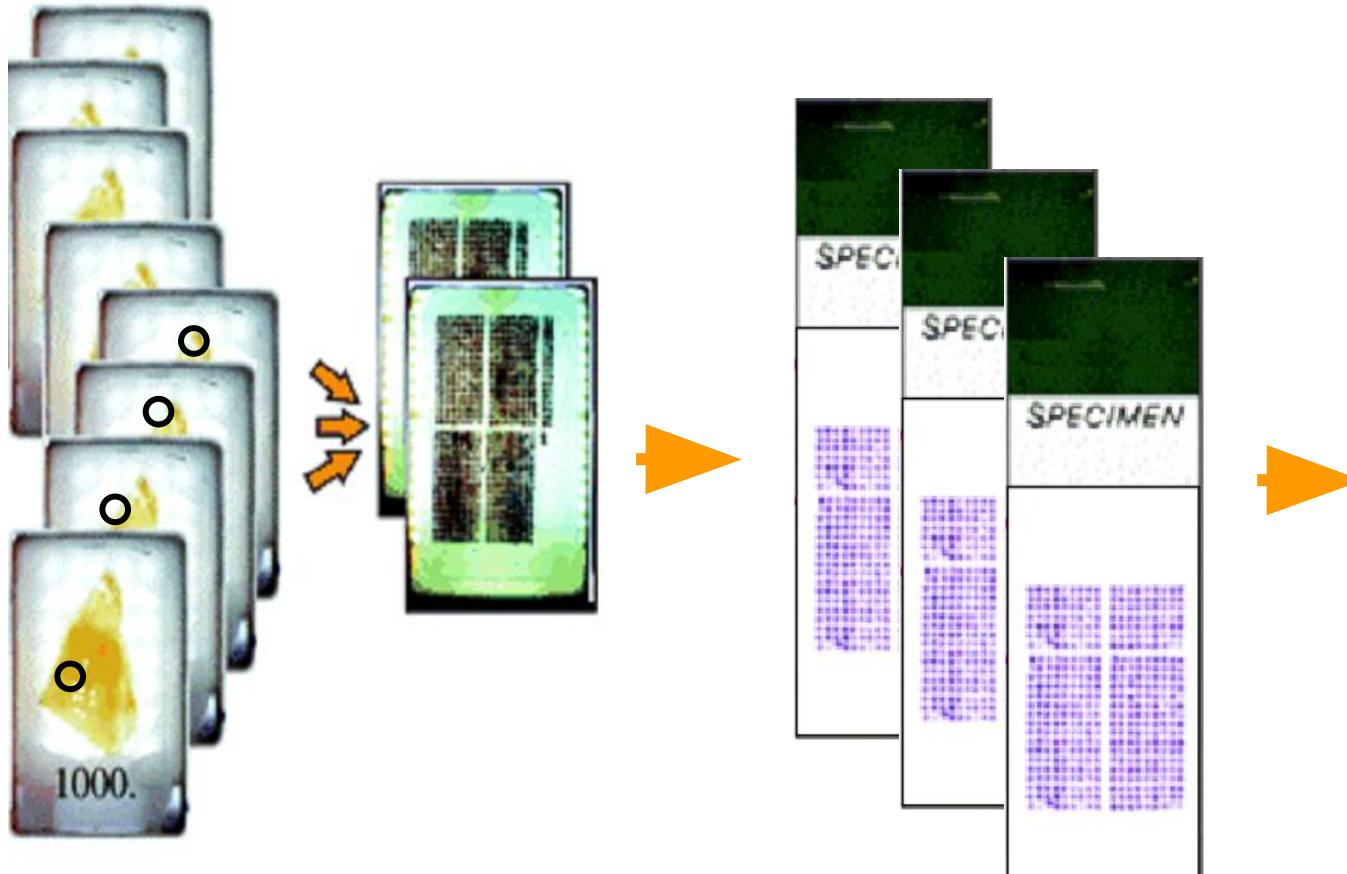
Donor block (Spinal cord)

Beecher Instruments  
Arrayer



Recipient block

# TMA<sup>s</sup> are constructed from multiple donor samples



Up to 1000 donors

Assembly into arrays

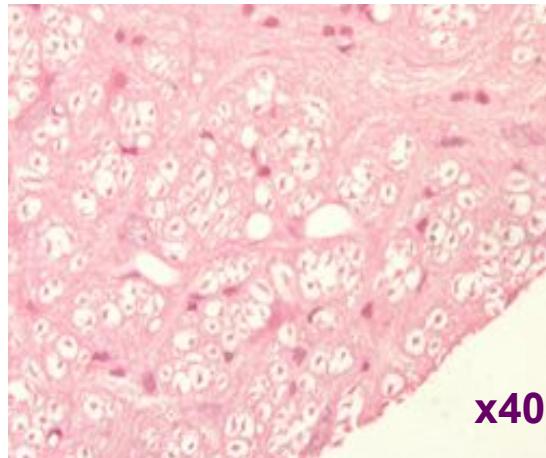
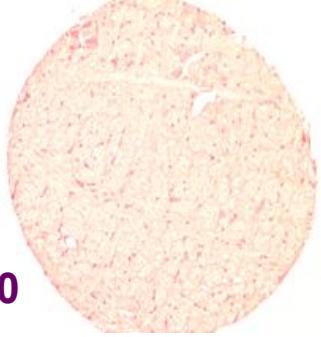
Up to 300 sections/array

**Analysis**  
Pathology  
Morphology  
Protein  
mRNA

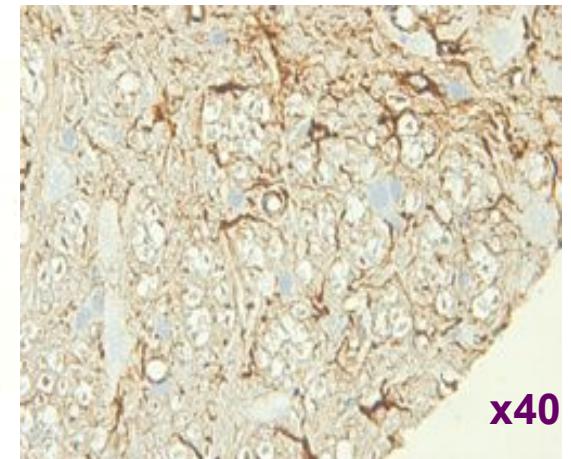
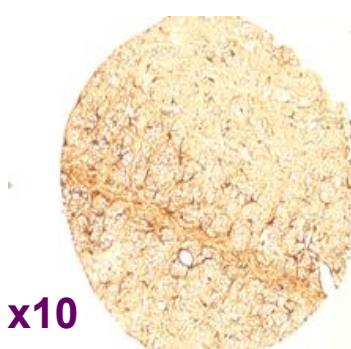
# Evaluation of TMAs

# *To determine biopsy accuracy and tissue quality*

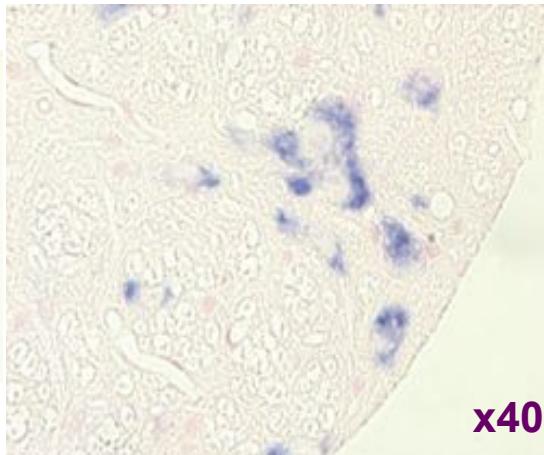
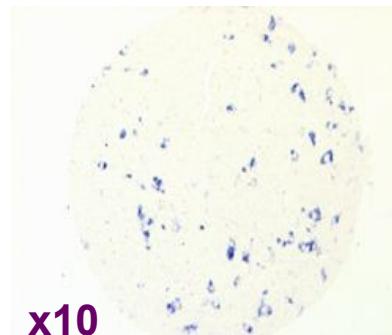
## Morphology Eosin/HTX



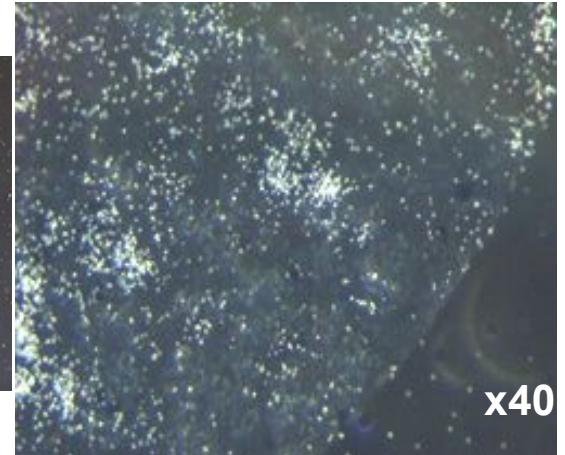
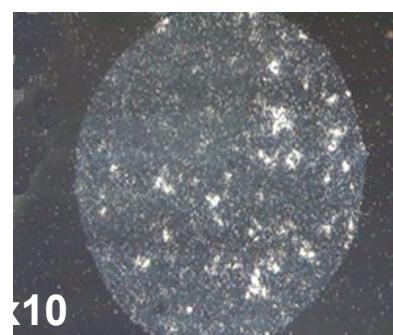
## Protein IHC (GFAP)



## mRNA Dig-ISH GAPDH



## mRNA Radiak-ISH GAPDH



# Application of TMA technology

## - "Normal" tissue arrays,

- Evaluation of antibody/and cRNA probe specificity
- Target distribution
- Splice variant expression
- Target related toxicology

## - Disease focused arrays,

- Expression profile of target to disease
- Individual variations, age, gender...
- Biomarker evaluation

## - Antibody screening arrays,

- Screening of antibodies

## - Cross-disease arrays,

- Secondary/alternative indications

# Proteomics



Sample preparation

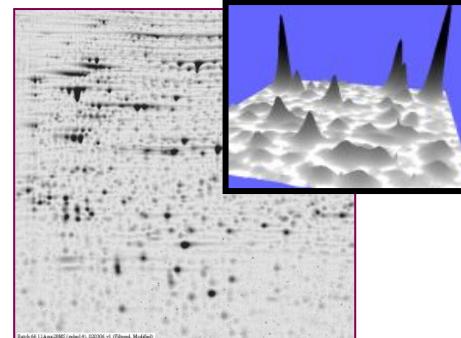
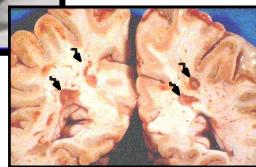
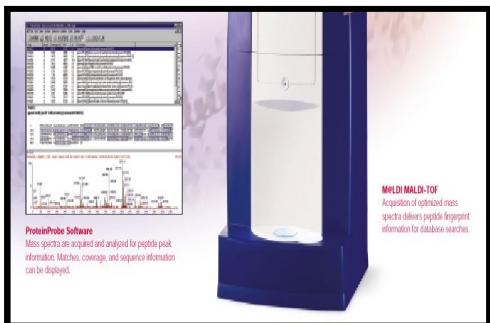


Image Analysis

2-D gel electrophoresis



Bioinformatics

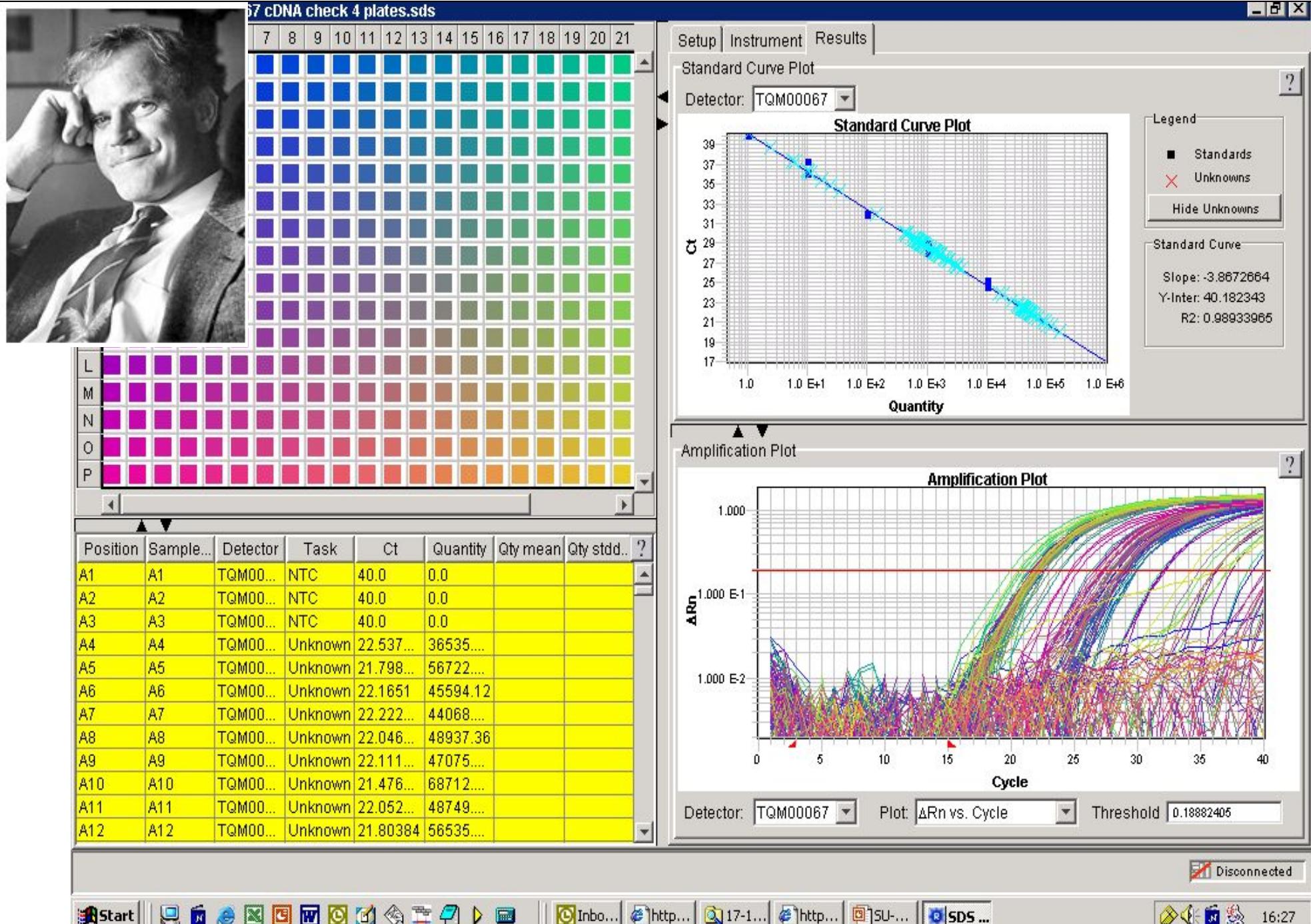


Mass Spectrometry

# Genechip and next generation sequencing technologies



# qPCR technologies





## Target validation

### Expression association to disease

+

Target must be present during disease  
Can get kinetics of disease progression

-

Expression not always increased or decreased  
during disease progression

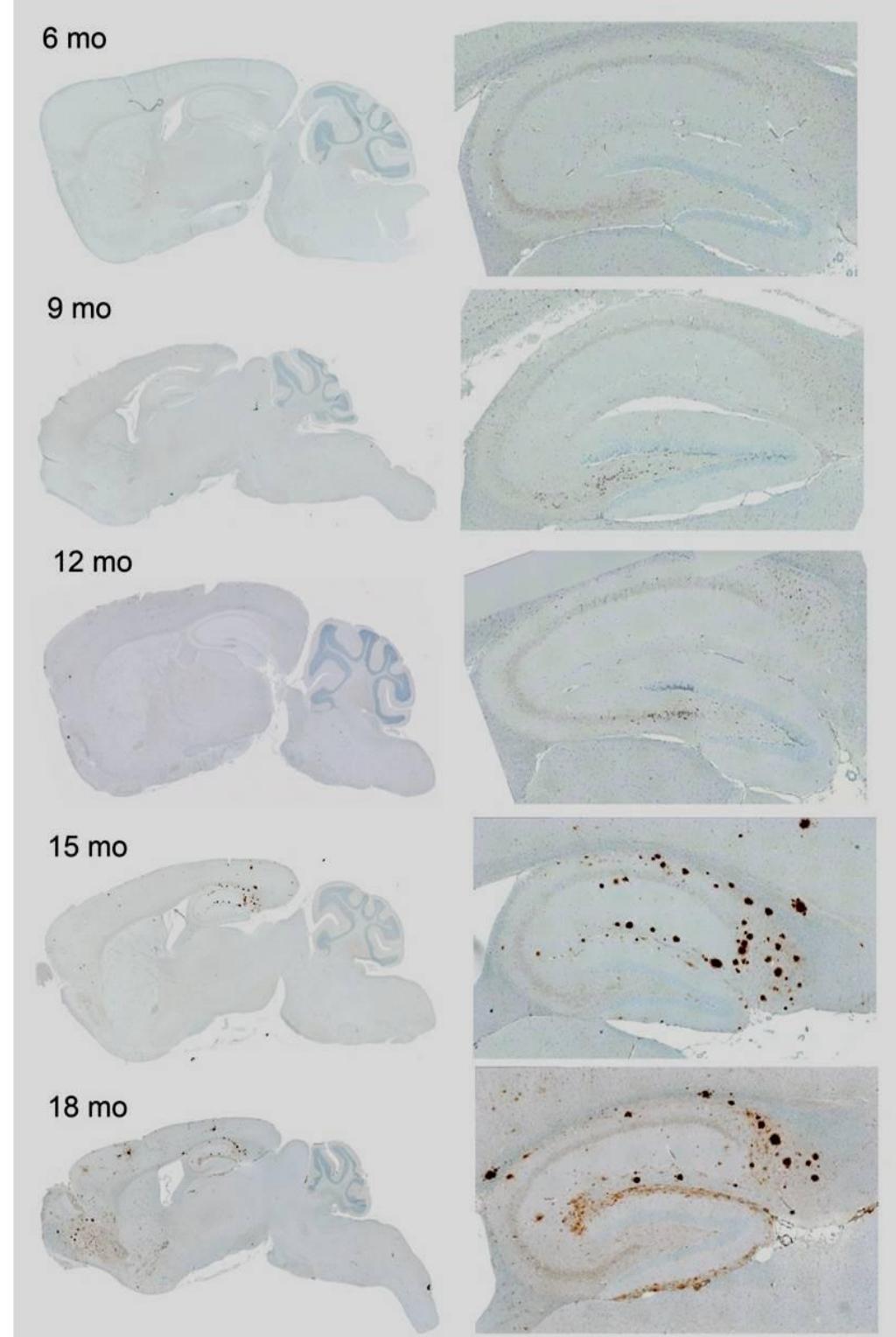
Is the expression a cause or a consequence of the disease?



## Animal disease models

transgenes:

- transgene *Tg2576* mice overexpressing human APP
- amyloid plaques
  - A $\beta$ , IHC





## Animal disease models, KOs

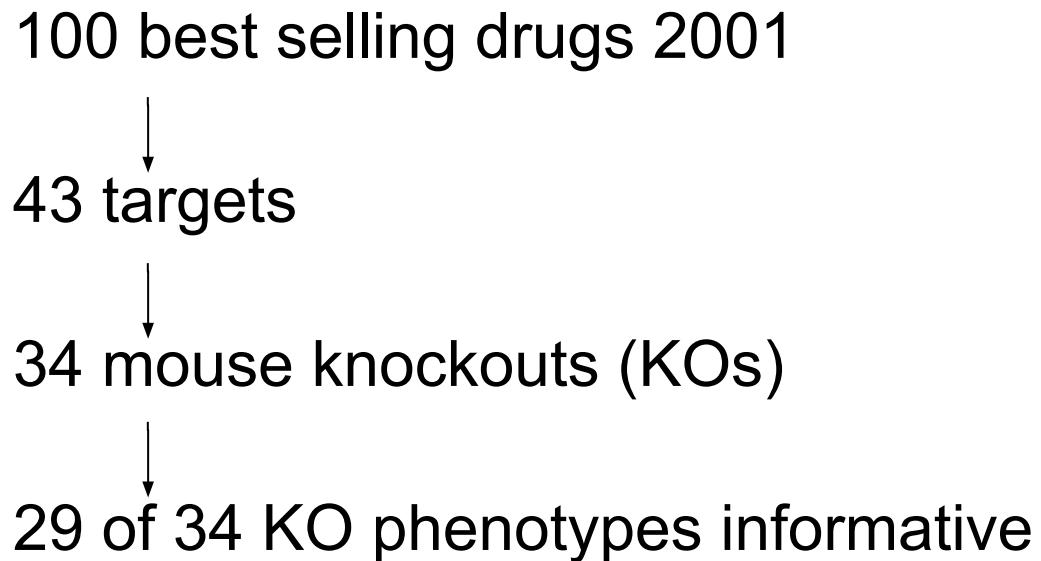


Mario R. Capecchi

Sir Martin J. Evans

Oliver Smithies

The Nobel Prize in Physiology or Medicine 2007 was awarded jointly to Mario R. Capecchi, Sir Martin J. Evans and Oliver Smithies "for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells".



Zambrowicz and Sands, Nature Reviews Drug Disc., 2, 38 (2003)



# Target validation

## Animal disease models

### Mouse knockout issues:

Embryonic lethality

Gene compensation

Differences between mouse and human physiology

**Still:** 29/34 KO phenotypes were informative in terms of gene function and pharmaceutical utility

Drug target	Drug name	Mouse phenotype
H <sup>+</sup> /K <sup>+</sup> ATPASE αKO βKO	Prilosec	pH 7 of stomach contents
		pH 7 of stomach contents
Serotonin transporter	Prozac	Altered open-field behavior



# Target identification / validation

Animal disease models, transgenes - KOs

+

Correlate well with drug effects

-

Time and resource consuming (Crispr/Cas faster)

Traditional KOs = on / off models (Crispr/Cas)

Embryonic lethality

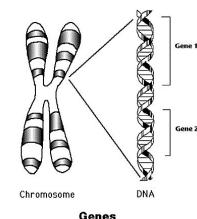
Gene compensation

Differences between mouse and human physiology

# Target validation



Literature study



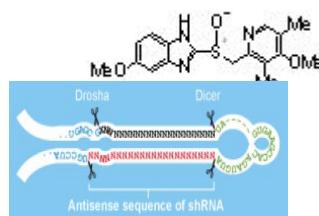
Genetic association to disease



Expression association to disease



Disease models



Compounds

- Small molecules
- Biopharmaceuticals
- RNA-based

# Validation using RNAi

nature  
neuroscience

## Targeting BACE1 with siRNAs ameliorates Alzheimer disease neuropathology in a transgenic model

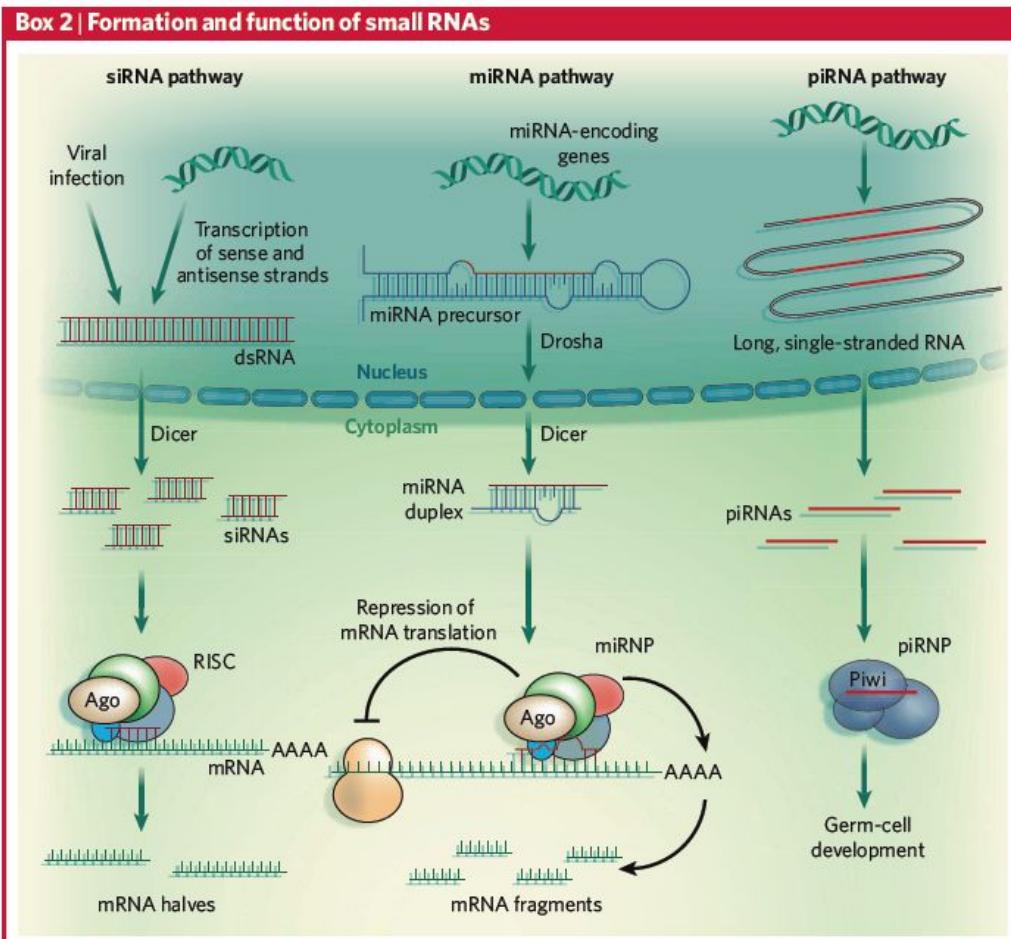
Oded Singer<sup>1,4</sup>, Robert A Marr<sup>1,4</sup>, Edward Rockenstein<sup>2</sup>, Leslie Crews<sup>2</sup>, Nicole G Coufal<sup>1</sup>, Fred H Gage<sup>1</sup>, Inder M Verma<sup>1</sup> & Eliezer Masliah<sup>2,3</sup>

In Alzheimer disease, increased  $\beta$ -secretase (BACE1) activity has been associated with neurodegeneration and accumulation of amyloid precursor protein (APP) products. Thus, inactivation of BACE1 could be important in the treatment of Alzheimer disease. In this study, we found that lowering BACE1 levels using lentiviral vectors expressing siRNAs targeting BACE1 reduced amyloid production and the neurodegenerative and behavioral deficits in APP transgenic mice, a model of Alzheimer disease. Our results suggest that lentiviral vector delivery of BACE1 siRNA can specifically reduce the cleavage of APP and neurodegeneration *in vivo* and indicate that this approach could have potential therapeutic value for treatment of Alzheimer disease.

Singer *et al.*, Nat Neurosci. **10**, 1343 (2005)

# Validation using RNAi

Box 2 | Formation and function of small RNAs



Andrew Z. Fire



Craig C. Mello

Großhans & Filipowicz, Nature (2008)

# Validation using lentiviral RNAi

forward: gatccGCAACGTATGGCAGGACAA<sup>t</sup>ca...agagaTTGTCTGCCATACGTTGCTttttggaaa

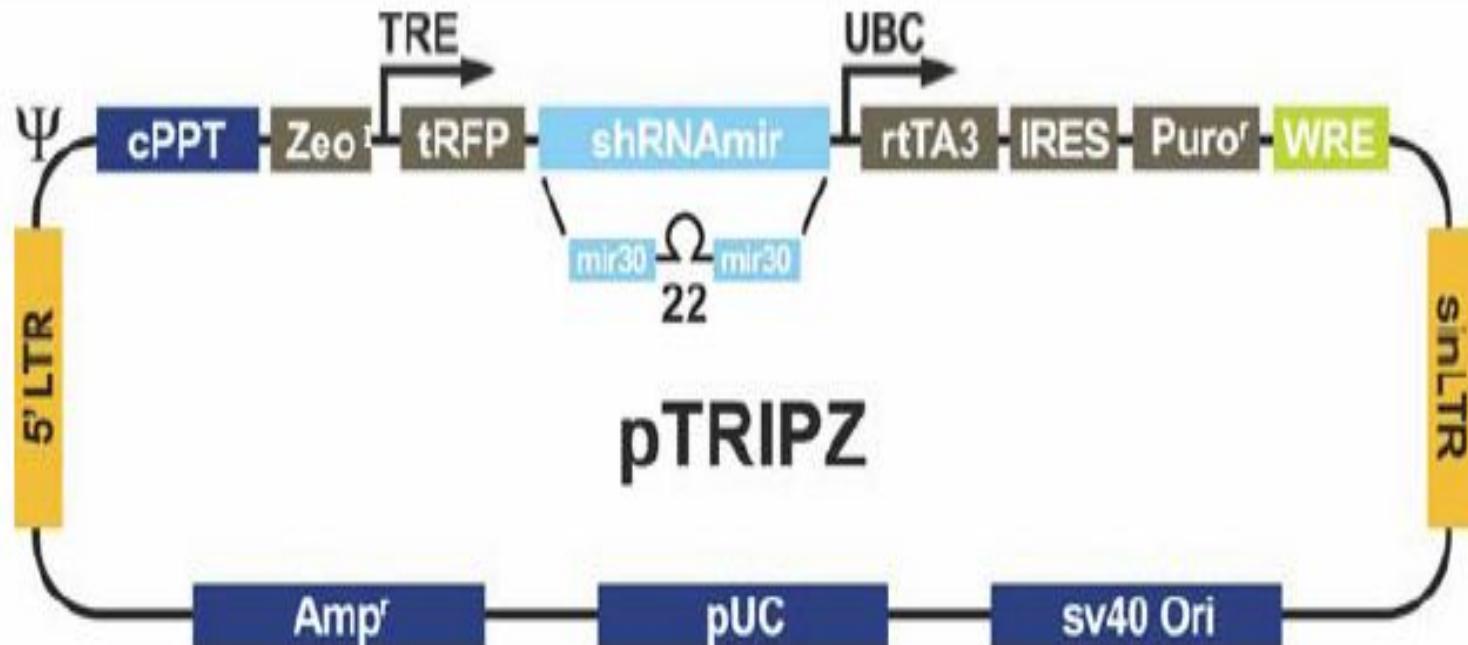
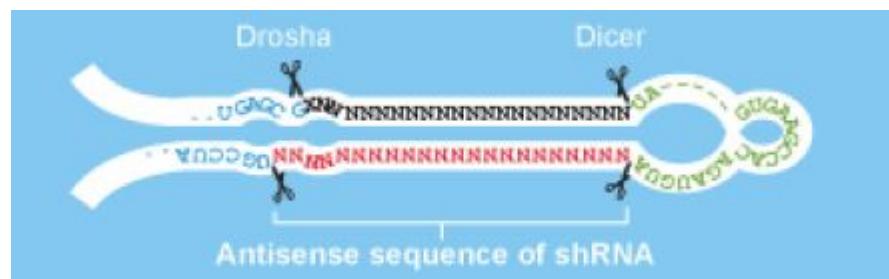
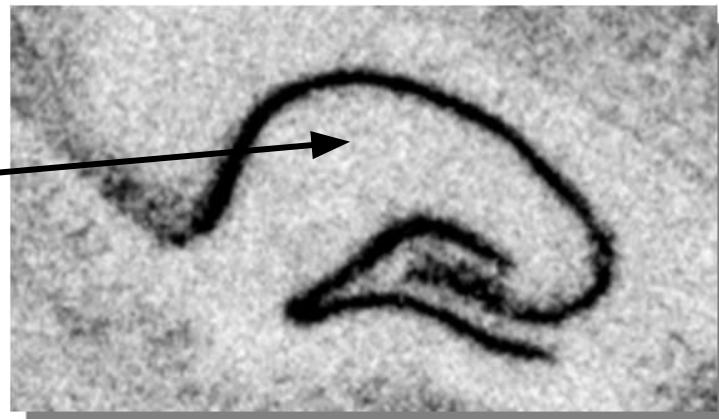
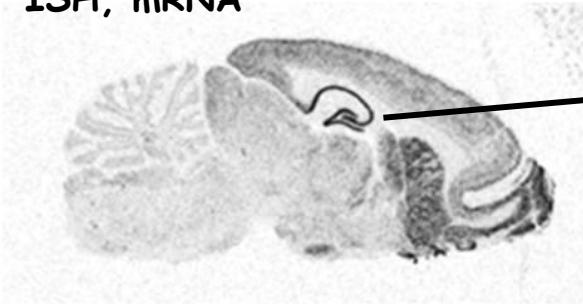


Figure 2. pTRIPZ lentiviral vector

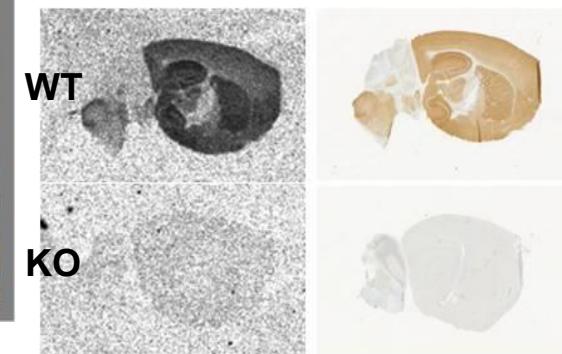
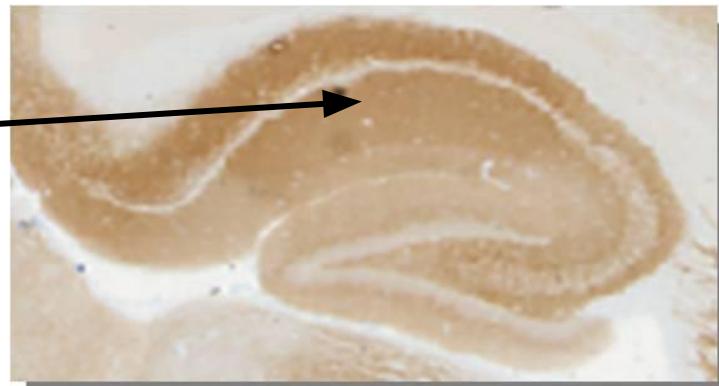
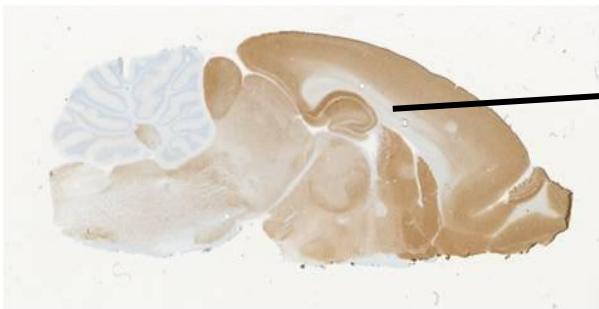


# Validation of compounds and targets

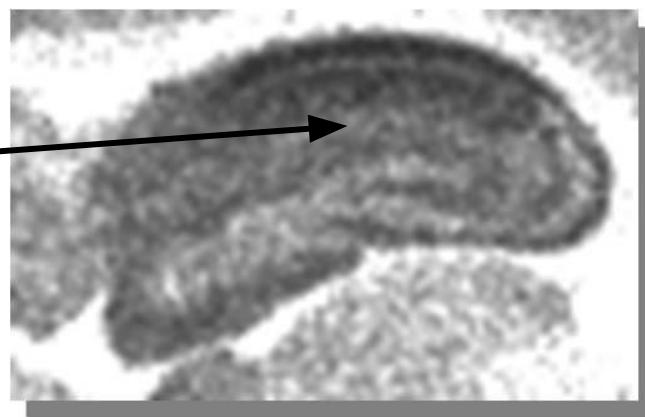
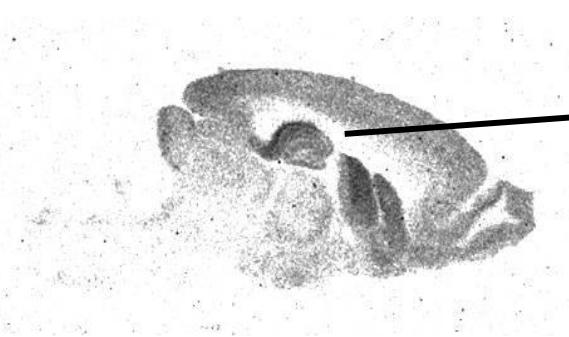
ISH, mRNA



IHC, Protein



[<sup>3</sup>H]CD Ligand Binding



# Target Validation – All data comes together

